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Selectivity and Steric Effects of Metoprolol Isomers on Isolated Rabbit Atria, Arteries and Tracheal Muscles

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ABSTRACT

Toda, Noboru, Shigehiro Hayashi, Yoshio Hatano, Hideki Okunishi and Mizuo Miyazaki: *Selectivity and steric effects of metoprolol isomers on isolated rabbit atria, arteries and tracheal muscles.* J. Pharmacol. Exp. Ther. **207:** 311–319, 1978.

Chronotropic and inotropic responses of isolated rabbit right and left atria to isoproterenol and also the relaxation induced by isoproterenol of tracheal muscles contracted with acetylcholine were antagonized by (−)-metoprolol to a greater extent than the response to isoproterenol of pulmonary arterial strips contracted with prostaglandin $F_{2\alpha}$. The pA_2 of (−)-metoprolol in right and left atria, tracheal strips and pulmonary arteries were 7.81, 7.79, 7.29 and 6.50, respectively. The pA_2 values of propranolol in these preparations were not appreciably different. In contrast, the response of isolated guinea-pig atria to isoproterenol was much more sensitive to metoprolol than tracheal and aortic strips, the pA_2 values being 7.93, 6.25 and 6.79, respectively. In rabbit atria and tracheal muscles, (−)-metroprolol was 270 to 380 times more potent than (+)-metoprolol in attenuating the response to isoproterenol, while in rabbit arteries, the former was approximately 20 to 30 times of the activity of the latter. (−)-Metroprolol attenuated the isoproterenol-induced relaxation of rabbit and dog coronary arteries to a greater extent than the relaxation of rabbit pulmonary and mesenteric arteries, the pA_2 values in the former arteries being almost identical with the values in rabbit atria. It may be concluded that susceptibility to selective *beta* antagonists of *beta* adrenoceptors of tracheal smooth muscles from different species differs; thus, the organ-selective action of the antagonists varies in different species. *Beta*-adrenoceptors in rabbit and dog coronary arteries appear to be $beta_1$.

The relative potency of a series of sympathomimetic amines for cardiac stimulation, fatty acid mobilization, bronchodilatation and vasodepression differs, and these responses can be classified into two groups following coefficients of correlation between the relative activities. Lands *et al.* (1967) have thus suggested that *beta* receptors are differentiated as $beta_1$ and $beta_2$. Practolol is the first $beta_1$- selective blocking agent introduced which antagonizes the cardiac stimulation induced by catecholamines to a greater extent than tracheal muscle relaxation and vasodilatation (Dunlop and Shanks, 1968; Barrett *et al.*, 1968). Recently, metoprolol has also been demonstrated to possess such a cardioselective action (Åblad *et al.*, 1973). In most studies, however, cardioselectivity has been analyzed in *in situ* or *in vitro* preparations (heart, airway smooth muscle and vasculature) from different species, or *beta* blocking actions on the heart have been compared with the actions either on the airway muscles or on vasculatures from same species.

The present study was thus undertaken to

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investigate the selectivity of *beta* blocking actions of (−)-metoprolol on isolated right and left atria, tracheal muscle and arteries from rabbits to determine relative potencies of (−)-, (+)- and (±)-metoprolol in these preparations, and to compare the antagonism by (−)-metoprolol of the response of coronary, pulmonary and mesenteric arterial strips to isoproterenol. *Beta* blocking actions of metoprolol were also compared in isolated rabbit and guinea-pig preparations.

Methods

Albino rabbits of both sexes, weighing 1.7 to 2.3 kg, were used. Under ether anesthesia, the animals were sacrificed by bleeding from carotid arteries. The heart, trachea, pulmonary artery and superior mesenteric artery were rapidly removed. The sinoatrial node-right atrial preparation and the left atrial preparation were prepared. Tracheal strips, approximately 20 mm in length, were prepared following the method described by Akçasu (1959) with minor modifications. Ventral interventricular branches of the left coronary artery were isolated from the heart. The coronary artery and the distal portion of pulmonary and mesenteric arteries were helically cut into strips, the lengths of which were approximately 20 mm (Furchgott, 1960). The right and left atrial preparations, tracheal muscle preparations and arterial strips were vertically fixed between hooks in the muscle bath of 20 ml capacity, containing the nutrient solution aerated with a mixture of 95% O_2 and 5% CO_2. Hooks anchoring the upper end of the preparations were connected to the lever of a force-displacement transducer (Sanei Sokki Co., Tokyo, Japan). Hooks fixing the lower end of left atria were connected to an electronic stimulator (Nihonkoden Kogyo Co., Tokyo, Japan). The solution was maintained at 30 ± 0.5°C for experiments with atria and at 37 ± 0.5°C for experiments with tracheal and arterial strips. These temperatures were adequate to obtain reproducible responses for 5 hr or longer. The resting tension was adjusted to 0.3 g for right atria, 3 g for left atria, 0.7 g for tracheal strips, 1.0 g for mesenteric and pulmonary arteries and 0.5 g for coronary arteries, these values except the value for right atria being optimal for inducing the maximum contraction. Constituents of the solution were as follows (millimolar concentrations): Na^+, 162.1; K^+, 5.4; Ca^{++}, 2.2; Mg^{++}, 1.0; Cl^-, 159.0; HCO_3^-, 14.9; and dextrose, 5.6. The pH of the solution was 7.2 to 7.3. Preparations were allowed to equilibrate for 90 to 120 min in control fluids, during which time the solutions were replaced every 10 to 15 min.

Isometric contractions and relaxations were recorded on an inkwriting oscillograph (Sanei Sokki Co.). Left atrial preparations were driven electrically by a train of 3 msec square pulses of supramaximum intensity (2–3 mA, 5 times threshold) applied at a frequency of 60/min (Toda, 1969). Dose-response curves of isoproterenol were obtained by adding the amine directly to bathing media in cumulative concentrations. The dose-response curves were reproducible after second series of experiments in the preparations used; therefore, the second dose-response curve was taken as a control. Tracheal, pulmonary arterial and mesenteric arterial preparations had been contracted with acetylcholine (5×10^{-7}–10^{-6} M), prostaglandin ($F_{2\alpha}$ (3×10^{-7}–10^{-6} M) and phenylephrine (10^{-7}–8×10^{-7} M), respectively, before isoproterenol-induced relaxations were obtained. The magnitude of contractions attained was 45 to 60% of the maximum contraction. At the end of experiments, maximum relaxations were obtained by the addition of 10^{-4} M papaverine, and isoproterenol-induced relaxations relative to those

TABLE 1

Mean pA_2 values of metoprolol isomers and (±)-propranolol in isolated rabbit atria, arteries and tracheal muscles

N, number of preparations used. Figures in parentheses indicate ranges of mean values ± S.E. Slope, slope of the plot of log (dose ratio − 1) *vs.* −log concentrations of antagonist. pA_2 values were calculated from this plot.

Preparations	(−) - Metoprolol				(+) - Metoprolol				(±) - Propranolol			
	N	pA_2	Slope	Conc. ratio[a]	*N*	pA_2	Slope	Isomeric ratio[b]	*N*	pA_2	Slope	Conc. ratio[a]
Right atrium	22	7.81	−1.09	1	12	5.23	−1.24	380	5	8.66	−0.96	1
Left atrium	7	7.79	−1.12	1.0	5	5.36	−1.04	270				
Coronary artery	5	7.60 (7.45–7.84)	−1.03	1.6								
Pulmonary artery	6	6.50 (6.40–6.62)	−1.13	21	11	4.99 (4.89–5.12)	−0.94	32	5	9.21 (9.10–9.35)	−1.20	0.3
Mesenteric artery	7	6.18 (6.03–6.42)	−0.95	43	6	4.86 (4.66–5.22)	−0.88	21				
Trachea	11	7.29 (7.19–7.40)	−0.93	3.3	14	4.82 (4.73–4.94)	−0.90	296	10	6.00 (7.92–8.09)	−0.97	4.3

[a] Concentration ratio: antilog [(pA_2 of (−)-metoprolol in right atria) − (pA_2 of (−)-metoprolol in other preparations)].
[b] Antilog [(pA_2 of (−)-isomer) − (pA_2 of (+)-isomer)].

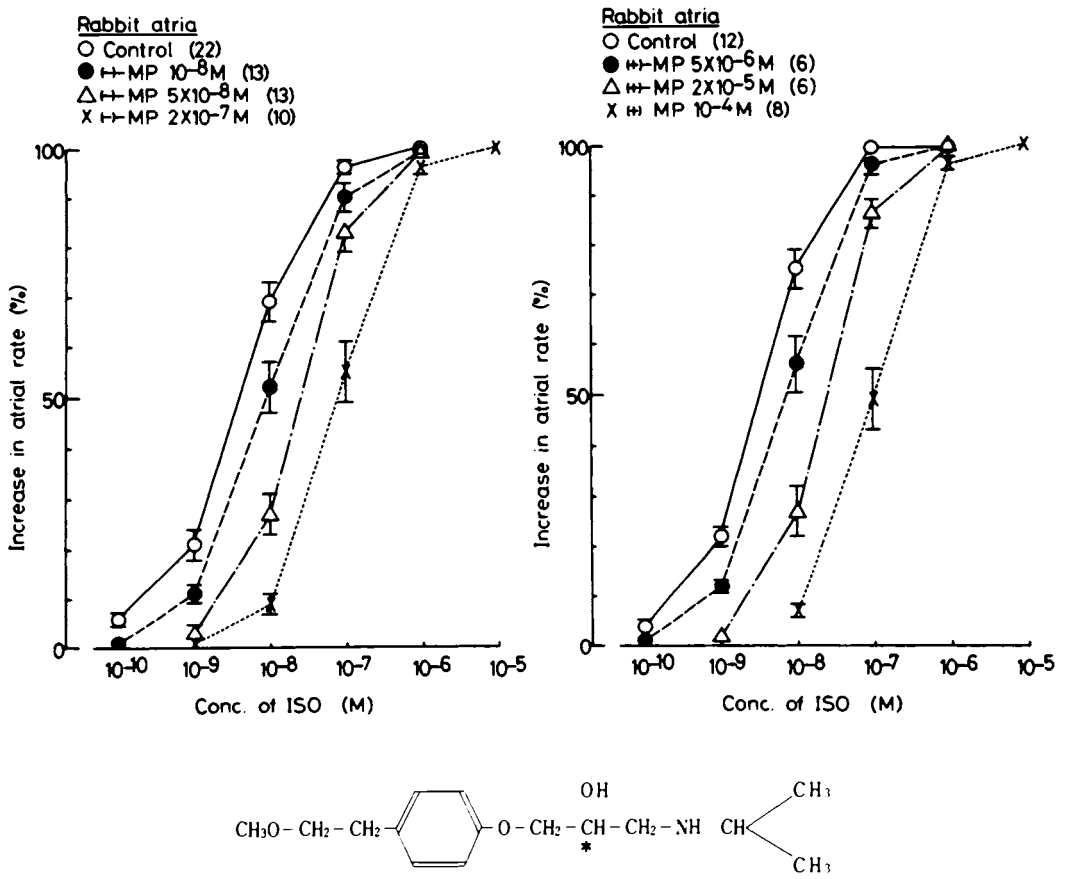

Fig. 1. Modification by (−)- and (+)-metoprolol of the positive chronotropic response of isolated rabbit right atria to isoproterenol. Vertical bars represent S.E.M. Figures in parentheses indicate the number of preparations used. Mean values of the atrial rate before the addition of the amine in control and (−)-metoprolol (10^{-8}, 5×10^{-8} and 2×10^{-7} M)-treated preparations were 110 ± 6 beats/min (N = 22), 110 ± 9 beats/min (N = 13), 109 ± 9 beats/min (N = 13) and 102 ± 10 beats/min (N = 10), respectively (for left figure), and those in control and (+)-metoprolol (5×10^{-6}, 2×10^{-5} and 10^{-4} M)-treated preparations were 110 ± 7 beats/min (N = 12), 115 ± 7 beats/min (N = 6), 101 ± 10 beats/min (N = 6) and 96 ± 8 beats/min (N = 8), respectively (for right figure). The maximum rate increase induced by isoproterenol was taken as 100%; mean absolute values in control and (−)-metroprolol (10^{-8}, 5×10^{-8} and 2×10^{-7} M)-treated atria were 94 ± 5 beats/min (N = 22), 103 ± 5 beats/min (N = 13), 93 ± 7 beats/min (N = 13) and 85 ± 8 beats/min (N = 10), respectively (for left figure), and those in control and (+)-metoprolol (5×10^{-6}, 2×10^{-5} and 10^{-4} M)-treated atria were 104 ± 12 beats/min (N = 12), 91 ± 14 beats/min (N = 6), 101 ± 12 beats/min (N = 6) and 105 ± 9 beats/min (N = 8), respectively (for right figure). The chemical structure of metoprolol is demonstrated. *Asymmetrical carbon.

induced by papaverine are presented in figures and the text. Preparations had been exposed for 30 min to blocking agents before the dose-response curve of isoproterenol was obtained. The pA_2 value was estimated from the ratio of median effective concentrations (ED50) of isoproterenol in the presence and absence of blocking agents (Arunlakshana and Schild, 1959). Only two doses of blocking agents could be used in same atrial preparations; therefore, mean pA_2 values were obtained by plotting mean values of log (dose ratio − 1) against log doses of antagonists.

Atrial preparations, tracheal muscle strips and aortic helical strips were also obtained from guinea pigs (350–400 g b.wt.). Helical strips of ventral interventricular branches of the left coronary artery from dogs (8–15 kg b.wt.) were prepared. The preparations were fixed in bathing media as described above. The resting tension was adjusted to 0.2 g for atria, 0.3 g for tracheal strips and 1.5 g for guinea-pig aortic strips and dog coronary arterial strips. Tracheal preparations were contracted with histamine (3×10^{-6}–10^{-5} M), and guinea-pig aortic strips and dog arterial strips were

contracted with prostaglandin $F_{2\alpha}$ ($2–8 \times 10^{-7}$ M). These stimulating agents produced 40 to 60% of the maximum contraction.

The results are expressed as mean values ± S.E.M. Comparisons of the results were made using the Student's *t* test. Drugs used were D(−)-, D(+)- and D(±)-metoprolol hydrochloride, (±)-propranolol hydrochloride, (±)-isoproterenol hydrochloride, prostaglandin $F_{2\alpha}$, acetylcholine chloride, histamine dihydrochloride and phenylephrine hydrochloride.

Results

Effects of metoprolol on isolated rabbit atria. The addition of (−)- and (+)-metoprolol in concentrations of 10^{-5} and 10^{-4} M slightly slowed the atrial rate: mean values of the rate decrease in response to the (−)-isomer were 5 ± 1 and 10 ± 3 beats/min ($N = 6$), respectively, from the predrug rate of 111 ± 8 beats/min, and the values with the (+)-isomer were 3 ± 1 and 6 ± 2 beats/min ($N = 5$), respectively, from the predrug rate of 114 ± 13 beats/min. The lower concentrations of metoprolol failed to alter the atrial rate.

The dose-chronotropic response curve of isoproterenol was shifted to the right by treatment for 30 min with (−)-metoprolol in concentrations higher than 10^{-8} M and by (+)-metoprolol above 5×10^{-6} M (fig. 1). Average pA_2 values of (−)-, (±)- and (+)-metoprolol against ED50 of isoproterenol were 7.81, 7.53 and 5.23, respectively (table 1). (±)-Propranolol was approximately 10 times more potent than (−)-metoprolol.

The additon of (−)- and (+)-metoprolol in concentrations up to 10^{-6} M failed to alter significantly the contractile force of left atria driven electrically at a rate of 60/min. The force was attenuated by metoprolol in concentrations of 10^{-5} and 10^{-4} M in a dose-dependent manner: average attenuations with the (−)-isomer were 7.8 ± 2.3 and 20.6 ± 2.8% ($N = 5$), respectively, from the predrug contraction of 2.39 ± 0.19 g, and the attenuations with the (+)-isomer were 7.3 ± 1.2 and 24.0 ± 2.1% ($N = 3$), respectively,

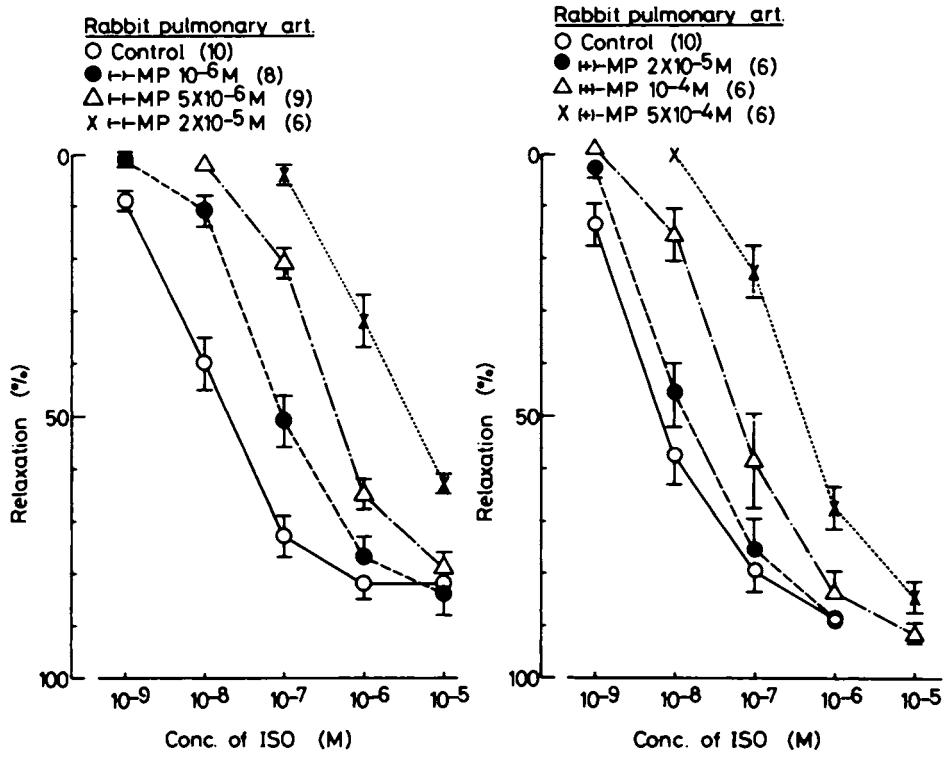

Fig. 2. Modification by (−)- and (+)-metoprolol (MP) of the relaxing response of rabbit pulmonary arterial strips to isoproterenol. The strips were contracted with prostaglandin $F_{2\alpha}$. Maximum relaxations induced by 10^{-4} M papaverine were taken as 100%; mean absolute values in control and (−)-metoprolol (10^{-6}, 5×10^{-6} and 2×10^{-5} M)-treated preparations were 523 ± 153 mg (N = 10), 686 ± 193 mg (N = 8), 599 ± 171 mg (N = 9) and 656 ± 219 mg (N = 6), respectively (for left figure), and those in control and (+)-metoprolol (2×10^{-5}, 10^{-4} and 5×10^{-4} M)-treated preparations were 855 ± 148 mg (N = 10), 980 ± 305 mg (N = 6), 911 ± 252 mg (N = 6) and 855 ± 211 mg (N = 6), respectively (for right figure).

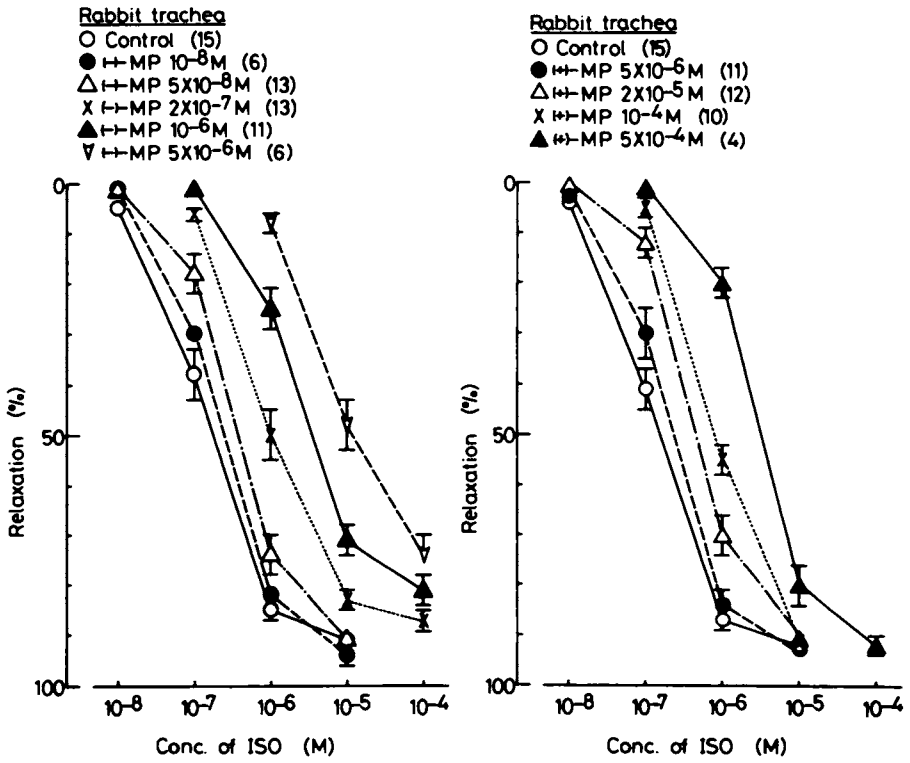

Fig. 3. Modification by (−)- and (+)-metoprolol (MP) of the relaxing response of rabbit tracheal strips to isoproterenol. The strips were contracted with acetylcholine. Maximum relaxations induced by 10^{-4} M papaverine were taken as 100%; mean absolute values in control and (−)-metoprolol (10^{-8}, 5×10^{-8}, 2×10^{-7}, 10^{-6} and 5×10^{-6} M)-treated preparations were 416 ± 41 mg (N = 15), 329 ± 30 mg (N = 6), 413 ± 43 mg (N = 13), 389 ± 38 mg (N = 13), 353 ± 35 mg (N = 11) and 309 ± 43 mg (N = 6), respectively (for left figure), and those in control and (+)-metoprolol (5×10^{-6}, 2×10^{-5}, 10^{-4} and 5×10^{-4} M)-treated preparations were 449 ± 51 mg (N = 15), 328 ± 34 mg (N = 11), 339 ± 49 mg (N = 12), 299 ± 41 mg (N = 10) and 388 ± 21 mg (N = 4), respectively (for right figure).

from the predrug contraction of 1.60 ± 0.26 g. The minimum current intensity sufficient to elicit atrial contractions (0.58 ± 0.07 mA, $N = 6$) was not influenced by (−)-metoprolol up to 10^{-4} M in four out of four preparations nor by (+)-metoprolol in two out of two preparations. The functional refractory period obtained by increasing the stimulus frequency stepwise was not affected by metoprolol in concentrations lower than 10^{-5} M but was slightly prolonged at 10^{-4} M (−)- and (+)-metoprolol. Mean values of the minimum interstimulus interval to obtain the equal magnitude of contractions in response to all stimuli before and after treatment with the (−)-isomer were 354 ± 52 and 385 ± 42 msec (N = 4), respectively, and those before and after treatment with the (+)-isomer were 342 ± 63 and 381 ± 57 msec ($N = 3$), respectively.

The positive inotropic effect of isoproterenol was significantly attenuated by treatment with (−)-metoprolol in concentrations of 10^{-8} M or higher and by (+)-metoprolol above 5×10^{-6} M. Avearge pA_2 values of (−)-, (±)- and (+)-metoprolol were 7.79, 7.34 and 5.36, respectively (table 1).

Effects of metoprolol on isolated rabbit arteries. Helically cut strips of pulmonary and superior mesenteric arteries were neither contracted nor relaxed by (−)- and (+)-metoprolol in concentrations up to 10^{-4} M. In prostaglandin $F_{2\alpha}$-contracted arterial strips, metoprolol above 10^{-5} M produced a slight contraction.

In pulmonary arteries contracted with prostaglandin $F_{2\alpha}$(3×10^{-7}–10^{-6} M), the addition of isoproterenol in concentrations ranging from 10^{-9} to 10^{-6} M caused a dose-related relaxation. Treatment for 30 min with (−)-metoprolol (10^{-6}–2×10^{-5} M) shifted the dose-response curve of isoproterenol to the right in a dose-dependent manner, whereas only at 10^{-4} M (+)-isomer were relaxations induced by isoproterenol significantly attenuated (fig. 2). Average

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