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Adrenoceptor Blocking and Cardiovasucular Effects of the Optical Isomers of Amosulalol (YM-09538), a Combined α- and β-Adrenoceptor Blocking Agent, and the Corresponding Desoxy Derivative (YM-11133) in Rats

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Abstract—The pharmacological activities of the enantiomers of amosulalol (YM-09538), a combined α- and β-adrenoceptor antagonist, and the corresponding desoxy derivative (YM-11133) were investigated in the cardiovascular system of rats. The optical isomers of amosulalol and YM-11133 antagonized the vasopressor effect of phenylephrine and the positive chronotropic effect of isoproterenol in normotensive pithed rats. Based on DR_2 values (μg/kg, i.v.) obtained from Schild plots, (+)-amosulalol and YM-11133 (DR_2=30) were approximately 10 times more potent than (−)-amosulalol (DR_2=324) in blocking α_1-adrenoceptors. For β_1-adrenoceptors, in contrast, (−)-amosulalol (DR_2=107) was approximately 60 times more potent than (+)-amosulalol (DR_2=6460), which was almost equipotent with YM-11133 (DR_2=3250). The results indicate that the optical isomers of amosulalol interact differently with α_1- and β_1-adrenoceptors. The effects of these phenethylamines on blood pressure and heart rate were studied in urethane-anesthetized rats (i.v.). The rank order of hypotensive potency in anesthetized rats ((+)-=desoxy>(−)-form) was consistent with the rank order of α_1-adrenoceptor antagonism in pithed rats. In contrast, (−)-amosulalol having a more potent β_1-adrenoceptor antagonist activity than (+)-amosulalol and YM-11133 only produced dose-dependent bradycardia at the hypotensive doses. The results indicate that the vascular α_1- and cardiac β_1-adrenoceptor blocking activities of the optical isomers of amosulalol contribute to their hypotensive and bradycardia, respectively. Thus, the racemate of amosulalol appears to exert an overall activity reflecting the activities of the individual isomers.

Amosulalol (YM-09538), a sulfamoyl-phenethanolamine derivative, is a newly developed antihypertensive agent with combined α- and β-adrenoceptor blocking activities (1, 2). In conscious spontaneously hypertensive rats (SHR), renal hypertensive rats and DOCA/salt hypertensive rats, a single oral administration of amosulalol (3–30 mg/kg) lowered acutely systolic blood pressure with a duration of over 6 hr. Repeated oral administration of amosulalol 50 mg/kg, b.i.d., for 12 weeks produced not only an antihypertensive effect without evidence of tolerance, but also reductions in plasma renin activity and heart rate in SHR with established hypertension (3). In adrenoceptor blocking studies in isolated tissues of rats and guinea pigs (2, 4) and in radioligand binding studies (5), amosulalol displayed a two orders of magnitude greater selective antagonism of α_1-adrenoceptors than α_2-adrenoceptors but amosulalol showed no selectivity towards β_1- or β_2-adrenoceptors. In guinea pig vascular tissues, amosulalol blocked the actions of norepinephrine and isoproterenol at pre- and post-junctional

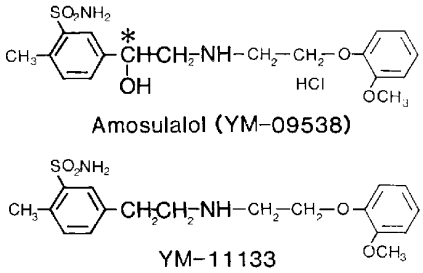

Fig. 1. Chemical structure of amosulalol (YM-09538) and the corresponding desoxy derivative (YM-11133). The asterisk denotes the point of asymmetry.

membranes without changing in the membrane properties of the smooth muscle cells. The inhibitory effects of amosulalol may be due to inhibition of α_1- and β-adrenoceptors. High concentrations of amosulalol had only weak inhibitory actions on α_2-adrenoceptors located in perivascular adrenergic nerve endings and in the smooth muscle membrane of the mesenteric vein (6). Amosulalol has one asymmetric center at the β-carbon (Fig. 1), so two enantiomers of this compound can exist. α- and β-Adrenoceptor subtype blocking activities of the optical isomers of amosulalol and the corresponding desoxy derivative (YM-11133) have been investigated in anesthetized rats (7), preliminarily, and in isolated tissues (8). (+)-Amosulalol and YM-11133 were one log unit order more potent and less potent than (−)-amosulalol in blocking α_1- and β_1-adrenoceptors, respectively, in both the anesthetized rat and the isolated tissue.

In this paper, postsynaptic vascular α_1-adrenoceptor and cardiac β_1-adrenoceptor antagonist activities and cardiovascular effects of the enantiomers of amosulalol and YM-11133 were investigated in rats in vivo.

Materials and Methods

Male normotensive Wistar rats (Shizuoka Agriculture Coop. Assoc., Shizuoka, Japan) weighing 280–330 g were used for evaluating the postsynaptic vascular α_1- and cardiac β_1-adrenoceptor antagonist activities and cardiovascular functions. The postsynaptic vascular α_1- and cardiac β_1-adrenoceptor blocking activities in pithed normotensive rats were assessed by antagonizing the α_1-adrenoceptor mediated pressor effect of phenylephrine and the β_1-adrenoceptor mediated positive chronotropic effect of isoproterenol, respectively. Cardiovascular functions were investigated in urethane-anesthetized rats (i.v.).

Adrenoceptor blocking activity: Wistar rats were anesthetized with ether and pithed by inserting a steel rod (1.5 mm in diameter) through the orbit and foremen magnum down into the spinal canal. Immediately after pithing, the animals were ventilated artificially with room air in a tidal volume of 1 ml/100 g body weight at a rate of 50 breaths/min using a rodent respirator (SN-480–7, Shinano, Tokyo, Japan). After bilateral vagotomy at the neck level, systemic arterial blood pressure was measured at the left carotid artery via a pressure transducer (MPU-0.5, Nihon Kohden, Tokyo, Japan) and recorded on a Nihon Kohden recorder (RJG-3004). Heart rate was measured with a cardiotachometer (RT-5, Nihon Kohden) triggered by pulse pressure. In the first series of experiments, the postsynaptic vascular α_1-adrenoceptor antagonist activity was studied after a 15 min period when cardiovascular parameters were allowed to stabilize. Phenylephrine was injected into the right femoral vein through a cannula at intervals of approximately 5 to 10 min until an increase in diastolic blood pressure of 80 to 120 mmHg. The dose-response curves for phenylephrine were constructed before and 15 min after i.v. treatment with each dose of antagonists. Three dose levels of antagonists given at an interval of approximately 1 hr were examined in the same animal. In a second series of experiments, the cardiac β_1-adrenoceptor antagonist activity was investigated in the other pithed rats. Dose-response curves for isoproterenol which was injected at an interval of approximately 5 to 15 min until an increase in heart rate of 80 to 100 beats/min were obtained before and 15 min after i.v. treatment with each dose of antagonists, and then three dose levels of antagonists were examined in the same animal as described above. The doses of antagonists were given at an interval of approximately 90 min. In

the study on both α_1- and β_1-adrenoceptor antagonist activities, both phenylephrine and isoproterenol administered in saline-treated rats (control study) repeatedly gave in 4 trials dose-response curves whose ED50 values were not significantly different from each other (data not shown). The ED50 values, doses of phenylephrine required to elicit a 50 mmHg increase in diastolic blood pressure and those of isoproterenol required to elicit a 50 beats/min increase in heart rate, were calculated from the log dose-response curves, and then the dose-ratio was calculated (3). The adrenoceptor antagonist activities were quantified by the method of Arunlakshana and Schild (9). The dose of antagonist required to produce an agonist dose-ratio of 2 (DR_2) and the slope of the regression line were calculated.

Study in anesthetized rats: Rats were anesthetized with urethane (1.2 g/kg, s.c.), and the vagal nerves were bilaterally cut at the neck level. The tracheas were cannulated to facilitate breathing, but the animals were permitted to respire spontaneously. Systemic arterial blood pressure and heart rate were measured as described above. Mean arterial pressure was calculated as the diastolic blood pressure plus one-third of the pulse pressure (mmHg). The test drugs were non-cumulatively injected into the right femoral vein through a cannula at an interval of 20 min and in a volume of 1 ml/kg.

Drugs: Amosulalol hydrochloride (YM-09538), its optical isomers and the corresponding desoxy derivative (YM-11133) were prepared by Dr. Fujikura in the Chemistry Department of Yamanouchi Pharmaceutical Co. The optical isomers were resolved into (−) and (+) tartaric acid salts of the enantiomers which were recrystallized to constant rotation in order to determine the absolute configuration and subsequently converted to hydrochloride salts. Physicochemical properties of the optical isomers were as follows:

R-(−)-Amosulalol: m.p. 158–160, $[\alpha]_D^{20}$ −30.4 (c=1, MeOH)

S-(+)-Amosulalol: m.p. 158–160, $[\alpha]_D^{20}$ +30.7 (c=1, MeOH)

(−)-Phenylephrine hydrochloride and (−)-isoproterenol hydrochloride (Tokyo Kasei, Tokyo, Japan), phentolamine methanesulfonate (Ciba-Geigy, Takarazuka, Japan), propranolol hydrochloride (Sigma Chemical Co., U.S.A.) were obtained commercially. Prazosin hydrochloride (Pfizer Inc., U.S.A.) was kindly donated by its manufacture. The enantiomers and racemate of amosulalol and the other drugs were dissolved in physiological saline (0.9 % w/v) but YM-11133 and prazosin were dissolved in a few drops of 1N HCl and distilled water, and then saline or 0.5% methylcellulose solution was added to them up to the appropriate volume. Ascorbic acid (0.01%, Takeda Chemical Industry, Japan) was added to the isoproterenol-containing solution to retard oxidation. Doses are expressed in terms of the salts, except for YM-11133, which was expressed as the base.

Data analysis: All results in the text are expressed as the mean±S.E.M. or the mean and 95% confidence limits with the number of experiments. Comparisons of values before and after the drug in the same group of pithed and anesthetized rats, and those of the vehicle and drug-treated group in conscious SHR were made by Student's paired and non-paired *t*-test, respectively. P values less than 0.05 are considered to be significant. Regression equations were calculated by the method of least squares.

Results

Adrenoceptor blocking effects

The basal diastolic blood pressure and heart rate were 55±2 mmHg and 305±5 beats/min (n=32), respectively, in pithed rats. ED50 values for phenylephrine and isoproterenol, doses of phenylephrine required to elicit a 50 mmHg increase in diastolic blood pressure and those of isoproterenol required to elicit a 50 beats/min increase in heart rate, were 2.7±0.2 μg/kg and 9.2±0.4 ng/kg (n=16), respectively, before treatment with antagonist. (+)-, (−)- and (±)-Amosulalol did not significantly (P>0.05) decrease basal blood pressure and heart rate.

Postsynaptic vascular α_1-adrenoceptor antagonistic activity: The log dose-response curves with respect to the increase in diastolic pressure elicited by phenylephrine, before and after pretreatment with (±)-amosulalol

(100, 300 and 1000 μg/kg, i.v.), (−)-amosulalol (300, 1000 and 3000 μg/kg, i.v.), (+)-amosulalol and YM-11133 (30, 100, 300 and 1000 μg/kg, i.v.) are shown in Fig. 2. (+)-, (−)- and (±)-Amosulalol and YM-11133 caused a parallel shift of the log dose-response curves for phenylephrine to the right. Based on DR_2 values (Table 1), (+)-amosulalol and YM-11133 were 10.7 and 10.9 times more potent than (−)-amosulalol in antagonizing phenylephrine-induced vasopressor response, respectively. In the same experimental condition, prazosin and phentolamine, α-adrenoceptor antagonists, also antagonized the phenylephrine-induced vasopressor effects with DR_2 values of 1.66 (n=12) and 128 (n=16) μg/kg, i.v., respectively, whereas propranolol hardly affected the log dose-response curves for phenylephrine up to 1 mg/kg, i.v. Both (+)-

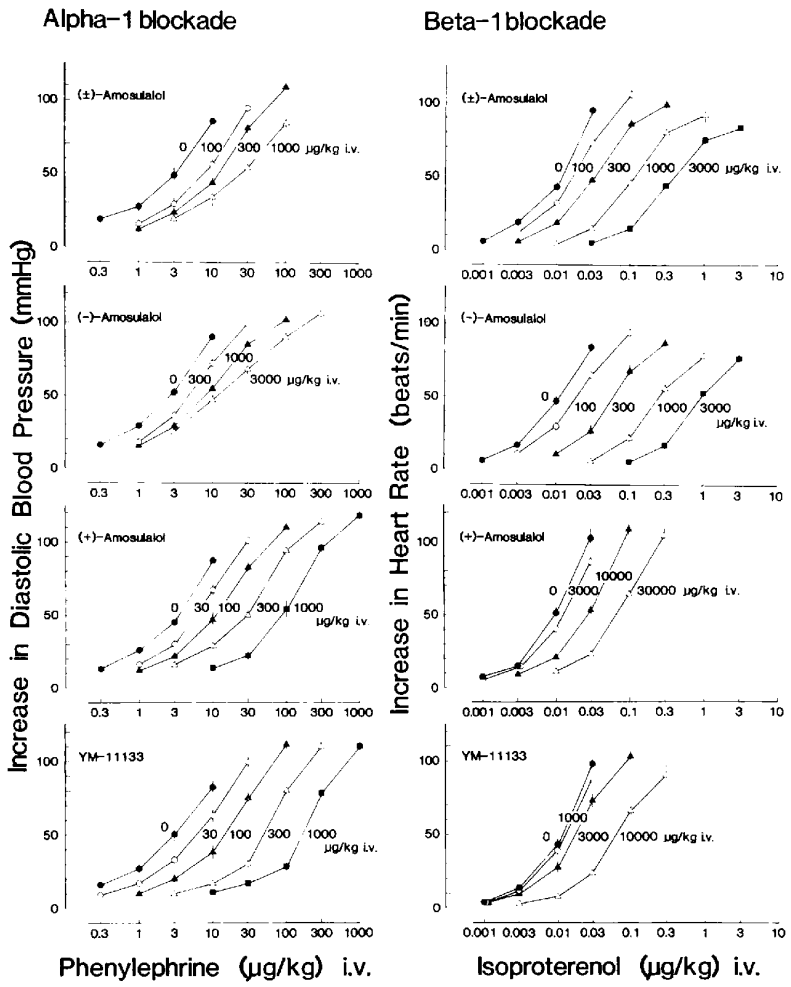

Fig. 2. Antagonism by (±)-, (−)- and (+)-amosulalol and the corresponding derivative (YM-11133) of phenylephrine-induced vasopressor effects (Left panel) and isoproterenol-induced tachycardiac effects (Right panel) in pithed rats. The results are the mean±S.E.M. of 4 animals.

Table 1. Alpha-1 and beta-1 adrenoceptor blocking activities of amosulalol, its optical isomers and the corresponding desoxy derivative (YM-11133) in pithed rats

	Alpha-1 blockade[a]				Beta-1 blockade[b]			
	n	DR_2[c]	Relative potency	Slope[c]	n	DR_2	Relative potency	Slope
(±)-Amosulalol	16	49.0 (25.1–97.7)	1/1.62	0.606 (0.299–0.913)	16	135 (110–166)	1/1.26	1.20 (1.01–1.31)
(−)-Amosulalol	12	324 (263–398)	1/10.7	0.621 (0.476–0.766)	16	107 (77.6–145)	1.00	1.41 (1.26–1.56)
(+)-Amosulalol	16	30.2 (21.4–42.7)	1.00	0.967 (0.805–1.13)	8	6460 (3020–13800)	1/60.4	1.24 (0.496–1.98)
YM-11133	16	29.6 (24.1–37.3)	1.02	1.18 (1.08 –1.28)	8	3250 (1960–5270)	1/30.4	1.64 (0.994–2.29)

[a]: Antagonism of phenylephrine-induced vasopressor effect. [b]: Antagonism of isoproterenol-induced tachycardiac effect. [c]: DR_2 values (μg/kg, i.v.) and slope of the Schild plot are the mean with 95% confidence limits in parentheses. n: Number of experiments.

amosulalol and YM-11133 were 18 times less but 4 times more potent than prazosin and phentolamine, respectively, in blocking vascular α_1-adrenoceptors; however, (−)-amosulalol was a weaker antagonist for α_1-adrenoceptors.

Cardiac β_1-adrenoceptor antagonistic activity: Figure 2 also illustrates the log dose-response curves for isoproterenol, before and after pretreatment with (−)- and (±)-amosulalol (100, 300, 1000 and 3000 μg/kg, i.v.), (+)-amosulalol (3000, 10000 and 30000 μg/kg, i.v.) and YM-11133 (1000, 3000 and 10000 μg/kg, i.v.). (−)- and (±)-Amosulalol dose-dependently produced a parallel shift of the log dose-response to isoproterenol to the right, but (+)-amosulalol and YM-11133 did not significantly cause a parallel shift to the right except the highest dose of 10000 μg/kg. Based on DR_2 values (Table 1), (−)-amosulalol, in contrast to its α_1-adrenoceptors, was 60.4 and 30.4 times more potent than (+)-amosulalol and YM-11133, respectively, in antagonizing isoproterenol-induced positive chronotropic response. Propranolol, a β-adrenoceptor antagonist, also antagonized isoproterenol-induced positive chronotropic effects with a DR_2 value of 28.2 μg/kg, i.v. (n=12), whereas prazosin did not cause any changes in the response to isoproterenol up to 1 mg/kg, i.v. (+)-Amosulalol, (−)-amosulalol and YM-11133 were 182, 3.8 and 94 times less potent, respectively than propranolol in antagonizing cardiac β_1-adrenoceptors.

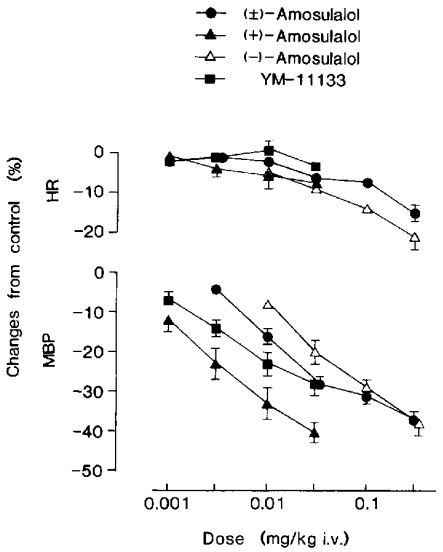

Fig. 3. Effects of (±)-, (−)- and (+)-amosulalol and the corresponding derivative (YM-11133) on mean blood pressure (MBP) and heart rate (HR) in urethane-anesthetized rats. The results are the mean±S.E.M. of 6–8 animals.

Study in anesthetized rats

Resting mean arterial pressure and heart rate of urethane-anesthetized rats were 114 ±4 mmHg and 459±9 beats/min (n=28),

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