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Cardiovascular effects of dl-nebivolol and its enantiomers – a comparison with those of atenolol

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In the present study, we investigated the cardiovascular effects of dl-nebivolol, a newly synthetized, chemically novel, β_1-adrenoceptor antagonist and its enantiomers, d-nebivolol (SRRR) and l-nebivolol (RSSS), in closed-chest anesthetized dogs, using atenolol as a reference substance. Results from preliminary studies in vitro indicate that d-nebivolol is the β_1-adrenoceptor antagonist and that l-nebivolol is practically devoid of β-adrenoceptor-blocking properties. Unlike atenolol, dl-nebivolol does not depress left ventricular function and slightly, but significantly, reduces peripheral vascular resistance over the dose range from 0.0025 to 0.04 $mg \cdot kg^{-1}$ i.v. These observations are likely to be clinically relevant because one daily oral dose of 5 mg dl-nebivolol effectively lowers arterial blood pressure in patients with hypertension. The favorable hemodynamic profile of dl-nebivolol can be ascribed to the l-enantiomer because the cardiovascular effects of this enantiomer are similar to those of the racemate. The cardiovascular profile of the d-enantiomer is similar to that of atenolol, albeit that its depressant effect on left ventricular function occurs at higher doses.

β_1-Adrenoceptor antagonists (selective); dl-Nebivolol; Atenolol; Left ventricular function; Closed-chest dog; (Enantiomers, Hemodynamics)

1. Introduction

dl-Nebivolol is a chemically novel, potent and selective β_1-adrenoceptor antagonist. (Van de Water et al., 1988). It is a racemic mixture of two enantiomers: d-nebivolol (SRRR) and l-nebivolol (RSSS) (fig. 1). dl-Nebivolol acutely lowers blood pressure in hypertensive humans and in spontaneously hypertensive rats. The compound decreases peripheral vascular resistance but does not depress, or even enhance, left ventricular function. Its selectivity in vivo is comparable to that of atenolol (Van de Water et al., 1988).

It was the aim of the present study to further investigate the hemodynamic profile of dl-nebivolol. Since the results of preliminary studies in vitro indicate that d-nebivolol is the β_1-adrenoceptor antagonist and that l-nebivolol has practically no β-adrenoceptor-blocking properties, the cardiovascular effects of both enantiomers were also investigated. Atenolol was used as a reference compound, because of its comparable selectivity in vivo. The study was performed on closed-chest anesthetized dogs.

2. Materials and methods

2.1. Experimental set-up and data aquisition

The experiments were performed on 28 mongrel dogs of either sex and varying age, ranging in

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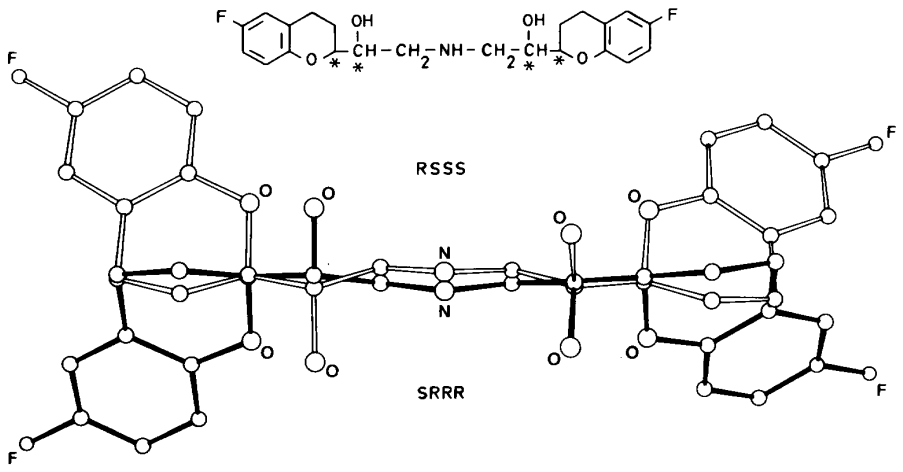

NEBIVOLOL

Fig. 1. Chemical structure and perspective drawing of dl-nebivolol. SRRR: d-enantiomer; RSSS: l-enantiomer.

body weight from 18 to 34 kg (median 27 kg). The dogs were divided into four groups. The animals received dl-nebivolol in one group (n = 7), d-nebivolol (n = 7) and l-nebivolol (n = 7) in the second and third group, respectively, and atenolol in the last group (n = 7). The animals were i.v. anesthetized with a mixture of scopolamine (0.015 $mg \cdot kg^{-1}$) and lofentanil (0.05 $mg \cdot kg^{-1}$) and intubated with a cuffed endotracheal tube. Intermittent positive pressure ventilation with a mixture of pressurized air and oxygen (60/40) was carried out with a volume-controlled ventilator (Siemens Elema). In the control period the CO_2 concentration in the expired air (ET CO_2), as determined with a capnograph (Gould Godart), was kept at 5% by adjustment of the respiratory volume (respiratory rate = 20 breaths $\cdot$ min^{-1}). A continuous i.v. infusion of etomidate (0.5 $mg \cdot kg^{-1} \cdot h^{-1}$) was started immediately after induction. Body temperature was monitored with a thermistor positioned in the pulmonary artery and kept at 37-38 °C. Heparin (1 000 IU $\cdot$ kg^{-1} i.v.) was administered to prevent the blood from clotting.

The electrocardiogram (ECG) was derived from limb leads (standard lead 2). Left ventricular (LVP) and ascending aortic blood pressure (AoP) were measured by retrograde catheterization via the femoral arteries with high fidelity catheter-tip micromanometers (PPG Biomedical Systems). Zero-line calibration was achieved by simultaneously recording the pressure signal through the lumen of the catheter with an external pressure transducer (Gould P23ID) positioned at the mid-chest level and equating both zero lines on the physiological recorder. A Swan-Ganz balloon guided thermistor catheter was placed via a femoral vein in the pulmonary artery to measure pulmonary artery blood pressure (PAP) with an external pressure transducer (Gould P23ID) positioned at the mid-chest level. The cardiac output (CO) was measured using the ECG (Snoeckx et al., 1976) and the respiration triggered thermodilution technique (Janssen Scientific Instruments). The other femoral vein was cannulated for injection of saline (at room temperature) into the right atrium and for injection of the test compounds. Peak ascending aortic blood flow velocity was measured through the right carotid artery with an electromagnetic catheter-tip probe connected to a square wave electromagnetic flow meter (Janssen Scientific Instruments). To continuously measure cardiac output (stroke volume times heart rate), calibration of the electromagnetic velocity catheter was accomplished by equating the area under the velocity

curve to the average stroke volume, calculated from the heart rate and cardiac output values. These values were assessed with the use of the thermodilution technique at the beginning of, and at various time intervals during, each experiment. All catheters were placed in position under fluoroscopic control. Continuous registration of the analog signals was performed on a 8-channel ink-jet recorder (Mingograph 800).

The blood pressure, thermistor, velocity and ECG signals were, via transducer amplifiers, fed into a digital minicomputer (PDP 11/23), connected to a dual diskette drive (RX02) and a display console (VT105). The following variables were calculated on-line and printed on a matrix printer (Facit 4512B), usually at 1 min intervals: heart rate (HR), the duration of the PQ, QRS and QT intervals, the QTc interval (QT interval corrected for heart rate with the Bazett formula, 1920), the R-wave amplitude, systolic (AoPs) and diastolic (AoPd) aortic blood pressure, systolic (PAPs) and diastolic (PAPd) pulmonary artery blood pressure, left ventricular end-diastolic pressure (LVEDP), the maximum positive rate of change of isovolumic LVP (LV dp/dt_{max}), this maximum first derivative divided by the actually developed pressure in the left ventricle (LV $dp/dt_{max}/Pd$), maximal aortic blood flow velocity (Aov_{max}) and acceleration (Ao dv/dt_{max}), cardiac output (CO), stroke volume (SV), and pressure rate product (PRP). The systemic vascular (SVR) and pulmonary vascular resistance (PVR) were calculated according to standard formulas. All values were simultaneously transmitted to a diskette and were plotted after the experiments on a digital x-y plotter (HP 7475A).

2.2. Protocol

dl-Nebivolol was dissolved in 10% hydroxypropyl-β-cyclodextrine ether in a concentration of 2.5 $mg \cdot ml^{-1}$ (pH 6.1). Its enantiomers were dissolved in the same solvent acidified with tartaric acid in a concentration of 1 $mg \cdot ml^{-1}$ (pH 2.5). Atenolol was dissolved in distilled water (concentration: 2.5 $mg \cdot ml^{-1}$; pH 8.1). The compounds were injected i.v. after a recorded control period of 20 min in cumulative doses of 0.0025, 0.01, 0.04, 0.16 and 0.63 $mg \cdot kg^{-1}$ at 30 min intervals.

2.3. Statistical analysis

The changes in the values of the various variables 1, 2, 5, 10, 20 and 30 min after administration of the various doses of the compounds (these values were compared to the control values, i.e. the median value of the values obtained just prior to, and 5 and 10 min before the first injection of the compound), were evaluated for statistical significance by applying the Wilcoxon m.p.s.r. test. Differences between control values and the results obtained with atenolol, dl-nebivolol and its enantiomers at various time intervals were evaluated for statistical significance by using Mann-Whitney's U-test. Two-tailed probabilities less than or equal to 0.05 were considered to be significant.

3. Results

3.1. Changes induced by dl-nebivolol

Cumulative i.v. administration of dl-nebivolol did not induce overt changes in the ECG. In the lower to median dose range (0.0025-0.04 $mg \cdot kg^{-1}$) the compound did not affect the pressure needed for the volume controlled respirator to ventilate the dog at a constant minute volume. The SVR decreased significantly and the CO and SV significantly increased following i.v. injection of 0.0025 and 0.01 $mg \cdot kg^{-1}$ dl-nebivolol. The AoPd decreased slightly, but significantly, starting after injection of 0.0025 $mg \cdot kg^{-1}$ i.v., while the LV $dp/dt_{max}/Pd$ increased slightly, but significantly, after administration of this dose. The PAPd, PVR and LVEDP increased significantly, starting after injection of 0.01 $mg \cdot kg^{-1}$ i.v. A significant decrease was noted in the HR after administration of 0.04 and 0.16 $mg \cdot kg^{-1}$ i.v. The LV dP/dt_{max} and PRP also decreased significantly following administration of 0.04 $mg \cdot kg^{-1}$ i.v., as did the LV $dP/dt_{max}/Pd$, Aov_{max}, Ao dv/dt_{max} and CO after 0.16 $mg \cdot kg^{-1}$ i.v., and the SV after the injection of 0.63 $mg \cdot kg^{-1}$ i.v. The SVR increased significantly after administration of 0.16 and 0.63

TABLE 1

The median values and 95% confidence limits of the hemodynamic variables before and after various doses of atenolol (A) and dl-nebivolol (N).

Variable	Drug	Control	Dose ($mg \cdot kg^{-1}$)				
			0.0025	0.01	0.04	0.16	0.63
Heart rate ($beats \cdot min^{-1}$)	N	75 (53-94)	72 (51-93)	71 (54-91)	65 [a] (45-88)	62 [a] (40-87)	68 (41-96)
	A	70 (55-110)	64 [a] (54-89)	61 [a] (50-96)	60 (44-104)	56 (41-115)	55 (40-118)
AoP syst. (kPa)	N	16.9 (16.3-19.9)	16.8 (16.3-19.6)	16.6 (16.1-19.2)	16.3 (15.2-18.7)	16.3 (14.5-17.5)	16.4 (13.9-17.7)
	A	16.1 (15.9-20.4)	15.7 [a,b] (14.8-20.4)	15.4 [a] (14.4-20.2)	15.1 [a] (13.1-20.1)	14.7 [a] (13.2-19.0)	14.1 [a] (12.1-19.0)
AoP diast. (kPa)	N	12.5 (11.6-14.3)	12.0 [a] (11.6-14.4)	11.8 [a] (9.9-14.3)	11.7 [a] (10.1-14.1)	12.0 (10.1-13.7)	12.0 [a] (9.9-13.7)
	A	10.8 (8.8-14.5)	10.4 [a] (8.3-14.5)	10.3 [a] (8.4-13.2)	10.0 [a] (8.1-13.3)	9.7 [a] (7.2-13.3)	9.3 [a] (6.0-13.0)
Cardiac output ($l \cdot min^{-1}$)	N	3.5 (2.0-6.9)	3.8 [a] (2.0-7.6)	3.7 [a] (2.1-6.8)	3.4 (1.9-5.8)	3.0 [a] (1.5-5.2)	2.3 [a] (1.2-3.8)
	A	4.1 (2.4-5.4)	3.7 [a,b] (2.3-5.0)	3.5 [a,b] (2.1-4.8)	3.1 [a,b] (1.8-3.5)	2.5 [a,b] (1.3-3.2)	2.6 [a] (1.4-3.2)
Stroke volume ($cm^3 \cdot beat^{-1}$)	N	48 (38-75)	55 [a] (39-85)	53 [a] (39-77)	54 (42-72)	45 (32-56)	36 [a] (31-42)
	A	55 (36-86)	51 [b] (31-77)	50 [b] (29-91)	44 [a,b] (25-80)	41 [a,b] (17-69)	39 [a] (20-63)
SVR ($kPa \cdot l^{-1} \cdot min$)	N	3.57 (2.32-6.63)	3.34 [a] (2.09-6.80)	3.24 [a] (2.35-6.39)	3.85 (2.75-5.47)	4.24 [a] (2.93-9.65)	5.29 [a] (4.04-11.29)
	A	3.05 (2.37-5.05)	3.28 [b] (2.33-5.60)	3.74 [a,b] (3.25-6.31)	4.56 [a,b] (3.16-7.31)	5.44 [a,b] (2.84-8.67)	5.73 [a] (2.45-7.80)
LV dp/dt_{max} ($kPa \cdot s^{-1}$)	N	301 (237-431)	297 (240-501)	292 (250-470)	261 [a] (202-336)	209 [a] (169-260)	190 [a] (139-209)
	A	322 (302-445)	290 [a,b] (286-414)	234 [a,b] (197-340)	192 [a,b] (166-305)	166 [a] (135-300)	159 [a] (116-245)
LV $dp/dt_{max}/Pd$ (s^{-1})	N	34 (29-48)	36 [a] (29-50)	35 (32-51)	33 (28-49)	26 [a] (22-41)	23 [a] (21-31)
	A	44 (36-53)	41 [a,b] (33-52)	38 [a,b] (28-52)	34 [a] (28-47)	32 [a] (26-40)	31 [a] (26-36)
LVEDP (kPa)	N	0.65 (0.29-0.87)	0.78 (0.07-0.99)	0.80 [a] (0.36-1.15)	0.96 [a] (0.35-1.89)	0.92 [a] (0.51-2.12)	1.08 [a] (0.71-1.36)
	A	1.05 (0.16-1.36)	1.29 (0.40-1.45)	1.48 [a] (0.85-1.91)	1.40 (0.88-2.01)	1.40 [a] (0.83-2.05)	1.70 [a] (1.03-2.13)
Aov_{max} ($cm \cdot s^{-1}$)	N	51 (41-67)	52 (48-69)	53 (46-68)	49 (33-76)	45 [a] (30-64)	42 [a] (25-26)
	A	56 (41-69)	53 [a,b] (37-85)	50 [a,b] (35-84)	47 [a,b] (30-73)	44 [a] (24-62)	43 [a] (22-57)
Ao dv/dt_{max} ($cm \cdot s^{-2}$)	N	1335 (1130-1907)	1408 (1264-2326)	1485 (1153-2529)	1385 (872-2608)	1085 [a] (685-1997)	860 [a] (553-1563)
	A	1695 (1413-2515)	1603 [a,b] (1344-1743)	1455 [a,b] (1128-1800)	1215 [a,b] (1022-2033)	945 [a,b] (898-1383)	844 [a] (561-1320)

TABLE 1 (continued)

Variable	Drug	Control	Dose ($mg \cdot kg^{-1}$)				
			0.0025	0.01	0.04	0.16	0.63
PRP	N	1333 (1053-1787)	1253 (867-1800)	1213 (907-1693)	1160 [a] (747-1640)	1080 [a] (680-1520)	1120 [a] (693-1573)
	A	1347 (920-1680)	1227 [a] (760-1547)	1160 [a] (787-1413)	1134 [a] (707-1693)	1054 (640-1547)	1014 [a] (587-1533)
PAP syst. (kPa)	N	2.5 (1.9-3.6)	2.6 (2.4-4.0)	2.8 (2.5-4.5)	3.3 (2.1-4.7)	2.9 (2.1-4.7)	2.8 (2.5-4.7)
	A	3.6 (2.3-4.4)	3.6 (2.1-4.0)	3.7 (2.0-4.0)	3.7 (2.7-4.0)	3.7 (2.5-3.7)	3.7 (2.8-3.9)
PAP diast.	N	0.7 (0.4-1.2)	1.0 (0.5-1.3)	1.1 [a] (0.5-1.5)	1.2 [a] (0.7-1.5)	1.2 [a] (0.8-2.1)	1.3 [a] (1.1-1.6)
	A	0.9 (0.3-1.5)	0.9 (0.3-1.5)	1.1 [a] (1.0-1.5)	1.2 (0.9-1.3)	1.2 (0.7-1.3)	1.2 (0.7-1.3)
PVR ($kPa \cdot l^{-1} \cdot min$)	N	0.37 (0.27-0.63)	0.41 (0.25-0.67)	0.45 [a] (0.28-0.69)	0.61 [a] (0.29-0.89)	0.69 [a] (0.33-1.16)	0.86 [a] (0.52-1.33)
	A	0.43 (0.25-0.83)	0.47 [a,b] (0.29-0.93)	0.58 [a,b] (0.37-0.99)	0.74 [a] (0.53-1.04)	0.76 [a] (0.64-1.44)	0.79 [a] (0.67-1.16)

[a] Significantly different from the control value; [b] significant difference between both groups.

$mg \cdot kg^{-1}$ i.v. No significant changes were observed in the AoPs and PAPs. The median values and 95% confidence limits of the haemodynamic variables before and after administration of various doses of dl-nebivolol are presented in table 1.

3.2. Changes induced by atenolol

The pressure needed for the volume-controlled respirator to ventilate the lungs of the animals at a constant minute volume did not change after administration of various doses of atenolol. A significant decrease was observed in the AoPs, AoPd, LV dp/dt_{max}, LV $dp/dt_{max}/Pd$, Aov_{max}, Ao dv/dt_{max}, CO, after all doses. The SV decreased significantly only at the higher doses (0.04 through 0.63 $mg \cdot kg^{-1}$ i.v.) and the HR only decreased in the lower dose range (0.0025-0.04 $mg \cdot kg^{-1}$ i.v.). The PRP decreased significantly after most of the doses injected. A significant increase was observed in the LVEDP, starting after i.v. injection of 0.01 $mg \cdot kg^{-1}$ atenolol. The SVR and PVR increased significantly after injection of 0.01 and 0.0025 $mg \cdot kg^{-1}$ i.v., respectively. The ECG showed a significant increase in the duration of the PQ interval, starting after 0.01 $mg \cdot kg^{-1}$ i.v., and of the QT interval following administration of the lowest dose. However, the QT interval did not show significant changes when corrected for the heart rate. The other measured or calculated variables did not show consistent, significant changes. The median values and 95% confidence limits of the variables before and after administration of doses of atenolol are presented in table 1.

3.3. Changes induced by d-nebivolol (SRRR)

The time intervals of the ECG did not change significantly after administration of this enantiomer, except for a slight, but significant decrease, in the duration of the QTc interval following administration of 0.63 $mg \cdot kg^{-1}$ d-nebivolol. A significant decrease was observed in the LV $dp/dt_{max}/Pd$ and Ao dv/dt_{max}, starting after i.v. injection of 0.01 $mg \cdot kg^{-1}$ and in the AoPs, Aov_{max}, SV and CO, starting after i.v. administration 0.16 $mg \cdot kg^{-1}$ of the compound. A significant increase was observed in the PAPd, PVR and SVR after i.v. injection of 0.01 and 0.04 $mg \cdot kg^{-1}$, respectively. These changes were also observed after i.v. injection of the remaining doses. The HR increased slightly, but significantly, after i.v. ad-

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