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New benzoxazine and benzothiazine derivatives — structure-activity relations in guinea-pig heart and smooth muscle preparations

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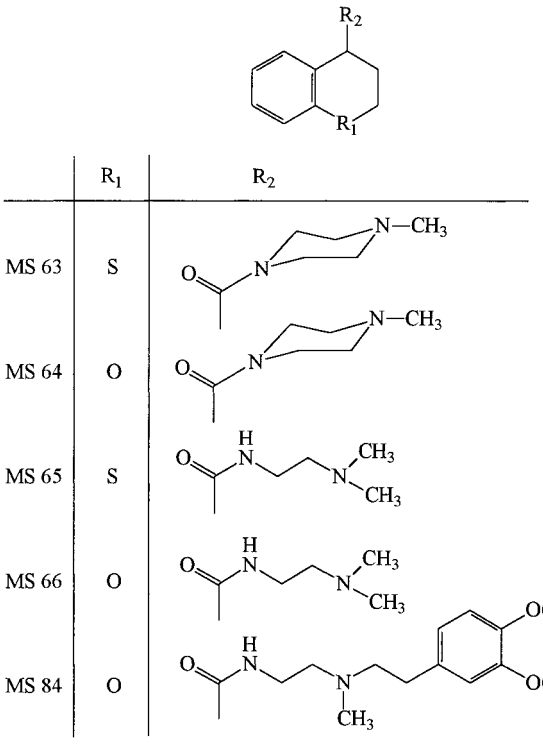
New benzoxazine and benzothiazine derivatives which differ in their side chains on the nitrogen atom of the benzoxazine or benzothiazine ring were investigated regarding structure-activity relations and compared with calcium antagonistic drugs. The isometric contraction force was measured in guinea-pig papillary muscles, aortic strips and terminal ilea. Chronotropic activity was studied in right atria of guinea pigs. The benzoxazine derivative with a dimethoxyphenylethyl-*N*-methylaminoethylcarboxamide side chain (MS 84) had the most potent negative inotropic effect on papillary muscles and the most potent relaxing effect on terminal ilea. The benzothiazine derivative with a methylpiperazinylcarbonyl side chain (MS 63) was less effective. Benzoxazine derivatives with a methylpiperazinylcarbonyl (MS 64) or a *N*-dimethylaminoethylcarboxamide side chain (MS 66) and the benzothiazine derivative (MS 65) with an analog side chain like MS 66 only had a weak effect. We conclude that the oxygen atom in the heterocyclic ring and the lipophilic side chain on the nitrogen atom, which is almost identical with the calcium antagonistic drug KT-362 is responsible for the most potent action.

1. Introduction

New benzoxazine and benzothiazine derivatives were investigated using guinea-pig heart muscle and smooth muscle preparations. The compounds differ in their substituents on the nitrogen atom in the molecule resulting in the compounds 3,4-dihydro-4[(4-methyl-1-piperazinyl)carbonyl]-2*H*-1,4-benzothiazine (MS 63), 3,4-dihydro-4[(4-methyl-1-piperazinyl)carbonyl]-2*H*-1,4-benzoxazine (MS 64), *N*-(2-dimethylaminoethyl)-3,4-dihydro-2*H*-1,4-benzothiazine-4-carboxamide (MS 65), *N*-(2-dimethylaminoethyl)-3,4-dihydro-2*H*-1,4-benzoxazine-4-carboxamide (MS 66) and *N*-[2-[*N*-[2-(3,4-dimethoxyphenyl)ethyl-*N*]-methylamino]ethyl]-2*H*-1,4-benzoxazine-4-carboxamide (MS 84).

These compounds also have structural similarities with calcium antagonists. The calcium antagonistic drug diltiazem shows a negative inotropic and chronotropic activity on isolated guinea-pig heart muscle preparations [1, 2]. The aim of this investigation was to study the drug profiles and structure-activity relations of the compounds and to compare them to those of calcium antagonistic drugs. Thus the inotropic, chronotropic and relaxing effects of MS 63, MS 64, MS 65, MS 66 and MS 84 were studied on isolated heart preparations, terminal ilea and aortic strips of guinea pigs.



2. Investigations and results

2.1. Effects on papillary muscles and right atria

The inotropic effect of the compounds was studied in isolated papillary muscles at a constant stimulation frequency of 1 Hz. MS 63 in a concentration range between 0.1 and 1000 μmol/l ($n = 4$) concentration-dependently reduced the force of contraction (f_c), time to peak force (t_1), relaxation time (t_2), maximum rate of force development (s_{1max}) and maximum rate of force relaxation (s_{2max}). At a concentration of 3 μmol/l MS 63 decreased f_c from 1.1 ± 0.3 to 0.54 ± 0.1 mN ($n = 4$, $P < 0.05$). MS 64 (1 to 100 μmol/l, $n = 4$) significantly decreased f_c, t_1, t_2, s_{1max} and s_{2max} in a concentration-dependent manner. MS 64 (30 μmol/l) reduced f_c from 1.2 ± 0.3 to 0.7 ± 0.2 mN ($n = 4$, $P < 0.05$). A concentration-dependent decrease of f_c was also caused by MS 65 (0.1–300 μmol/l, $n = 4$), MS 66 (0.3–1000 μmol/l, $n = 4$) and MS 84 (0.3–100 μmol/l). Additionally the compounds concentration-dependently reduced the other parameters of the contraction curve, except MS 65 and 84 that did not significantly change t_1 and t_2. MS 65 in a concentration of 10 μmol/l reduced f_c from 2.0 ± 0.5 to 0.9 ± 0.1 mN ($n = 4$, $P < 0.01$), MS 66 in a concentration of 3 μmol/l from 0.7 ± 0.1 to 0.5 ± 0.1 mN ($n = 5$, $P < 0.05$) and MS 84 in a concentration of 1 μmol/l from 1.8 ± 0.3 to 1.3 ± 0.2 ($n = 4$, $P < 0.05$). The effective concentrations for 50% reduction of the contraction amplitude (EC_{50}) are

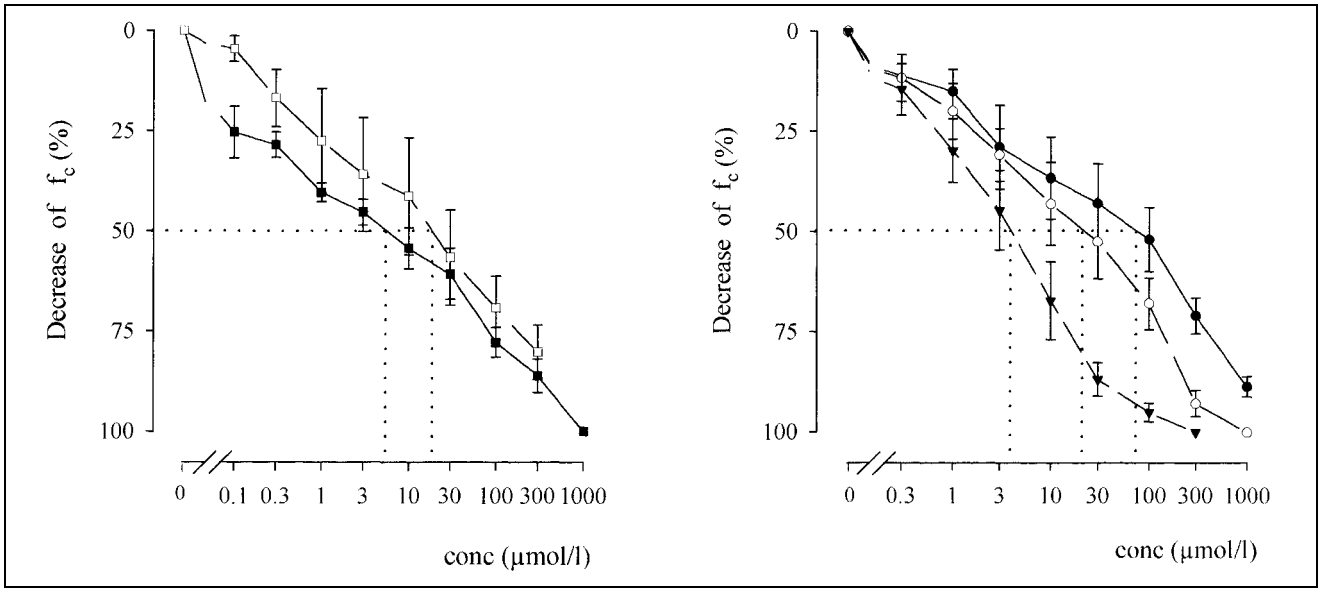


Fig. 1: The left panel shows the effect of MS 63 (■) and MS 65 (□), the right panel the effect of MS 64 (●), MS 66 (○) and MS 84 (▼) on isometric force of contraction (f_c) of guinea-pig papillary muscles. The decrease in percent of f_c is semilogarithmically plotted on the ordinate against the concentration of the compounds on the abscissa. Symbols represent the arithmetic means ± SEM from 4 experiments

presented in the Table. The decrease in percent of f_c of the compounds is shown in Fig. 1.

Spontaneously beating right atria were used to study the chronotropic effect of the derivatives. All compounds concentration-dependently reduced the rate of activity (Fig. 2). EC_{50} for MS 63, MS 64, MS 65, MS 66 and MS 84 are shown in the Table.

2.2. Effects on aortic strips

Aortic strips were stimulated with 90 mmol/l KCl. The relaxing effect of the compounds on contraction was studied in concentrations between 0.3 and 1000 µmol/l. The benzothiazine and benzoxazine derivatives concentration-dependently reduced f_c resulting in an EC_{50} of 99.0 ± 7.4 µmol/l for MS 63 (n = 3–5), 845 ± 22.9 µmol/l for MS 65 (n = 3) and 375 ± 18.5 µmol/l for MS 84 (n = 3–5). MS 64 (n = 2–4) and MS 66 (n = 4) did not show any effect up to 1000 µmol/l. The relaxing effects of the compounds on aortic strips are shown in Fig. 3.

Table: EC_{50} ± SEM for the negative inotropic effect (f_c) in papillary muscles and for the negative chronotropic effect (f) in right atria of guinea pigs

Compd.	EC_{50} of f_c (µmol/l)	EC_{50} of f (µmol/l)
MS 63	6.5 ± 1.2 (n = 4)	150.0 ± 14.5 (n = 4)
MS 64	84.0 ± 6.7 (n = 4)	500.0 ± 18.3 (n = 4)
MS 65	21.5 ± 3.5 (n = 4)	182.0 ± 12.8 (n = 4)
MS 66	24.0 ± 2.2 (n = 4)	570.0 ± 15.9 (n = 4)
MS 84	4.8 ± 1.0 (n = 4)	45.0 ± 6.4 (n = 4)

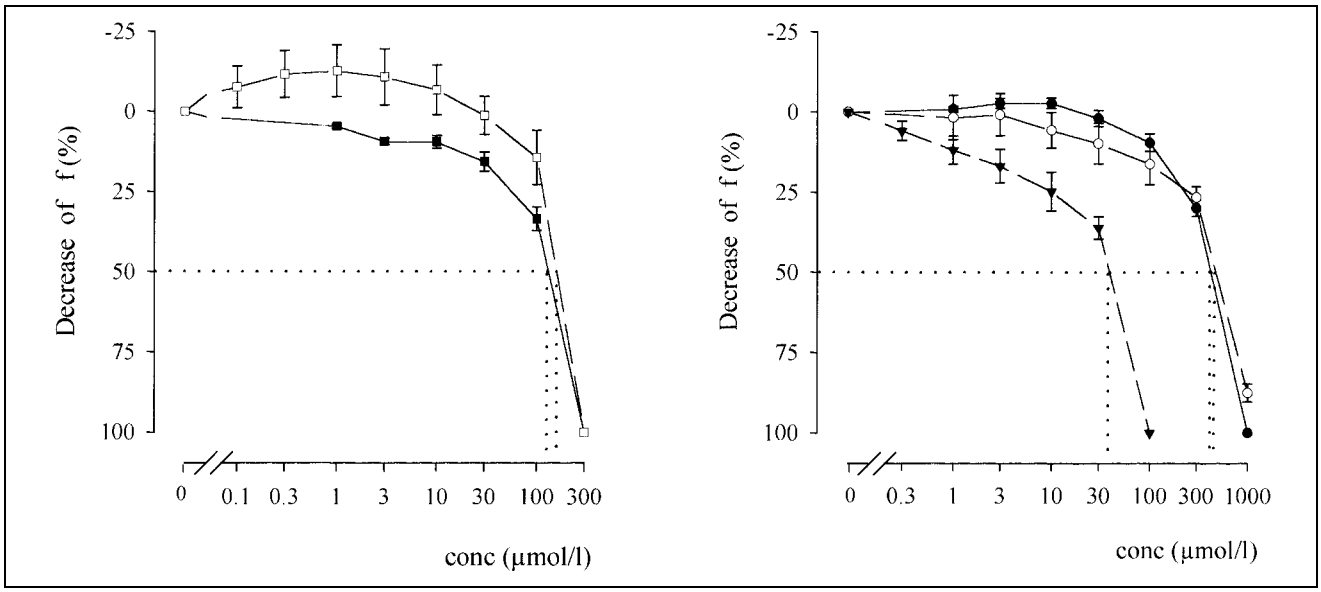


Fig. 2: The left panel shows the effect of MS 63 (■) and MS 65 (□), the right panel the effect of MS 64 (●), MS 66 (○) and MS 84 (▼) on spontaneous rate of activity (f) of guinea-pig right atria. The decrease in percent of f is semilogarithmically plotted on the ordinate against the concentration of the compounds on the abscissa. Symbols represent the arithmetic means ± SEM from 4 to 7 experiments

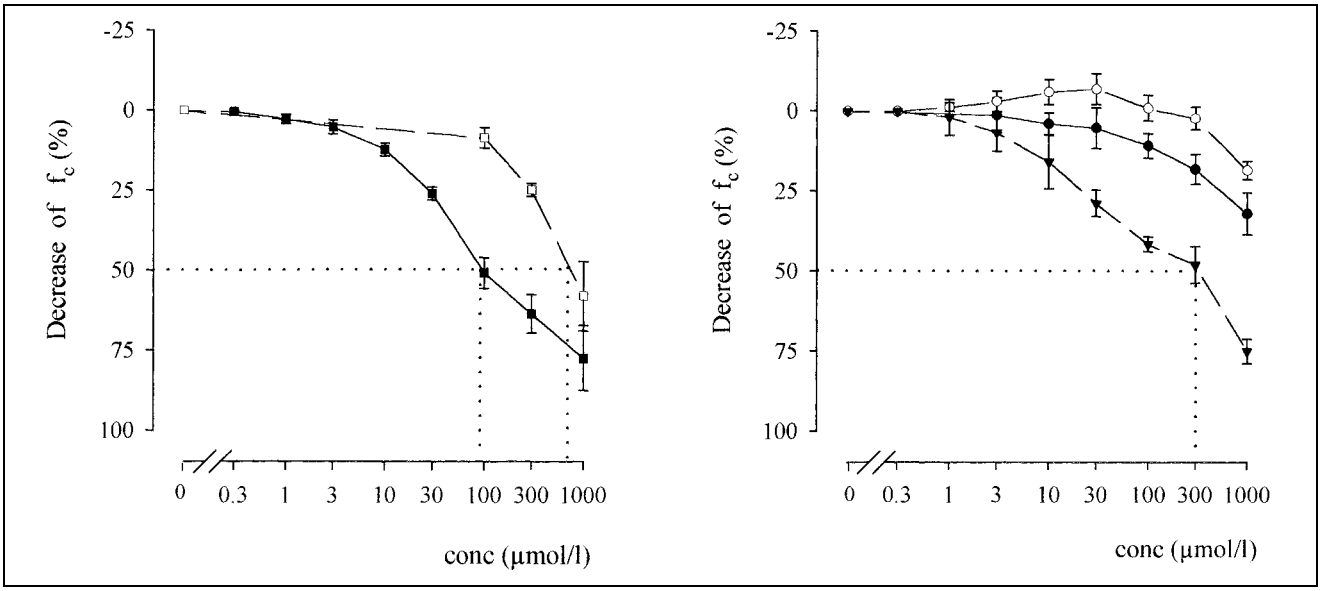


Fig. 3: The left panel shows the relaxing effect of MS 63 (■) and MS 65 (□), the right panel the effect of MS 64 (●), MS 66 (○) and MS 84 (▼) on aortic strips precontracted by 90 mmol/l KCl. The decrease in percent of contraction force is semilogarithmically plotted on the ordinate against the concentration of the compounds on the abscissa. Symbols represent the arithmetic means ± SEM from 3–5 experiments

2.3. Effects on terminal ilea

Similar experiments as on aortic strips were carried out using terminal ilea. The preparations were stimulated with 60 mmol/l KCl. Again the compounds concentration-dependently relaxed the KCl-induced contraction. The graphically estimated EC_{50}-values were 100 ± 11.2 μmol/l for MS 63 (n = 4), 261 ± 12.5 μmol/l for MS 65 (n = 4), 820 ± 20.2 μmol/l for MS 66 (n = 3) and 4.9 ± 1.5 μmol/l for MS 84 (n = 4). MS 64 (n = 4) had no effect up to a concentration of 1000 μmol/l. These data are shown in Fig. 4.

3. Discussion

The benzoxazine and benzothiazine derivatives investigated here differ in their side chain on the nitrogen atom. The benzothiazines MS 63 and MS 65 have a methylpiperazinylcarbonyl and a *N*-dimethylaminoethylcarboxamide side chain, respectively. The benzoxazines MS 64 and MS 66 have side chains analog to MS 63 and MS 65. MS 84 has a dimethoxyphenylethyl-*N*-methylaminoethylcarboxamide side chain. All compounds have structural similarities with calcium antagonistic drugs like diltiazem and KT-362. The EC_{50} for the negative inotropic effect of diltiazem is 16.1 μmol/l that is dependent on the extracellular potassium concentration [1]. The EC_{50} for KT-362 is 7 μmol/l [3]. In our studies MS 84 had the most potent negative inotropic effect with an EC_{50} of 4.8 ± 1.0 μmol/l, followed by MS 63 with an EC_{50} of 6.5 ± 1.2 μmol/l. The side chain of MS 84 is almost identical with that of KT-362. The only difference is that the benzoxazine derivative MS 84 has a tertiary amine structure in the side chain, whereas the benzothiazepine derivative KT-362 has a secondary amine structure. MS 63, MS 84 and KT-362 have

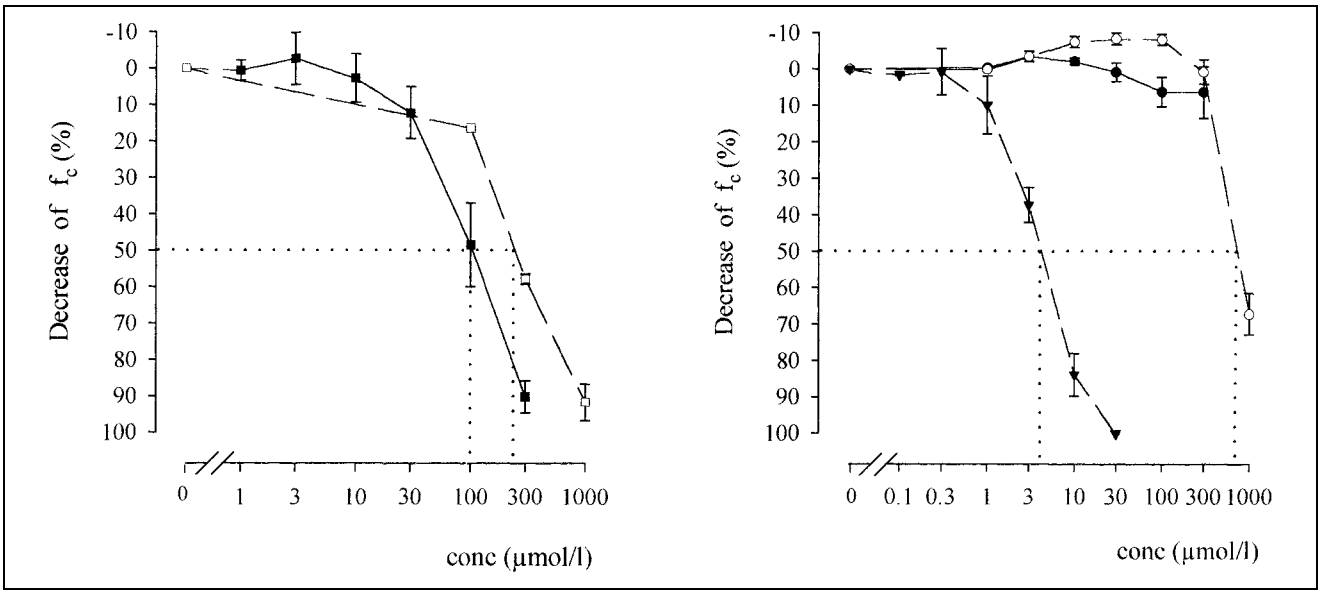


Fig. 4: The left panel shows the relaxing effect of MS 63 (■) and MS 65 (□), the right panel the effect of MS 64 (●), MS 66 (○) and MS 84 (▼) on terminal ilea precontracted by 60 mmol/l KCl. The decrease in percent of contraction force is semilogarithmically plotted on the ordinate against the concentration of the compounds on the abscissa. Symbols represent the arithmetic means ± SEM from 3–4 experiments

similar negative inotropic effects. Substitution of the sulfur atom in the MS 63 molecule by an oxygen atom results in the benzoxazine derivative MS 64 which exerts a less negative inotropic effect. Replacing the methylpiperazinylcarboxamide side chain in the MS 64 molecule by a *N*-dimethylaminoethylcarboxamide side chain (MS 66) does not change the negative inotropic effect on papillary muscles. Substitution of the oxygen atom in this compound results in the corresponding benzothiazine MS 65 with a negative inotropic effect similar to MS 64 and MS 66. Our results show that compounds with a sulfur atom in the heterocyclic ring (MS 63) and/or a lipophylic side chain (MS 63, MS 84) have the most potent negative inotropic effect. The decrease in activity is as follows: MS 84 > MS 63 > MS 65 ≥ MS 66 > MS 664. The negative chronotropic action of all derivatives studied was less pronounced than the negative inotropic action with the following activity MS 84 > MS 63 ≥ MS 65 > MS 64 > MS 66. Compounds with an oxygen atom in the heterocyclic ring and a lipophylic side chain (MS 64 and MS 84) had the most potent effect. To compare, the EC_{50} value for KT-362 was 23 μmol/l [3].

We also investigated the effect of the benzoxazine and benzothiazine derivatives on KCl precontracted terminal ilea and aortic strips. The most potent relaxing effect on terminal ilea showed MS 84. The action of the other compounds was less pronounced (MS 84 > MS 63 > MS 65 ≥ MS 66 > MS 64). The relaxing effect on aortic strips was at least 100 times weaker. The benzoxazine and benzothiazine derivatives only inhibited in high concentrations or failed to affect contractile responses to KCl precontracted aortic strips with the following potency: MS 63 > MS 84 > MS 65 > MS 64 and MS 66. It is believed that in vascular smooth muscle both potassium and calcium-induced contractions are primarily related to an increase in calcium influx through L-type calcium channels from the extracellular space as a result of membrane depolarization [4, 5]. KT-362 is an intracellular calcium inhibitor with antiarrhythmic and vasodilating effects [6, 7]. In isolated arterial vessel rings KT-362 inhibits the contractile responses to norepinephrine but not due to increased extracellular potassium [7]. The vasodilatory effect therefore might be due to an interference with the release of intracellular calcium [8].

Our results show that substitution of an oxygen atom by a sulfur atom in the basic structure results in a higher potency (MS 63, MS 64 and MS 65, MS 66, respectively). Increasing the lipophylicity in the side chain (MS 84) causes higher biological activity.

4. Experimental

4.1. Electromechanical studies on heart muscle preparations

Guinea pigs of both sexes (340–480 g) were killed with a blow on the neck. After excision of the heart, papillary muscles were dissected from the right ventricle for contractility measurements. Purkinje fibres were carefully removed to prevent spontaneous activity. Only muscles with a diameter of less than 0.87 mm were used in order to have a sufficient oxygen supply [5]. Chronotropic activity was tested on guinea-pig isolated right atria. The preparations were isolated and stored at room temperature in gassed (95% O_2 – 5% CO_2) Krebs-Henseleit solution with the following composition (in mmol/l): NaCl 114.9, KCl 4.73, $CaCl_2$ 3.2, $MgSO_4$ 1.18, $NaHCO_3$, 24.9, KH_2PO_4 1.18, glucose 10; pH 7.2–7.4. Isometric contraction force of electrically stimulated papillary muscles and spontaneous activity in right atria was measured by the method described by Reiter [9]. Experiments were performed at a temperature of 35 ± 1 °C. The bathing solution was continuously bubbled by a mixture of 95% O_2 and 5% CO_2 to guarantee sufficient oxygen supply and an appropriate pH as well as circulation of nutrient solution with and without test substance. A force transducer and amplifier (Transbridge™, 4-Channel Transducer Amplifier, World Precision Instruments, Sarasota, FL, USA) was used for the measurement of isometric contractions. Resting tension of either 3.92 mN (papillary muscles) or 10.37 mN (right atria) was kept constant throughout the experiments.

Papillary muscles were electrically driven with an Anapulse Stimulator Model 301-T and an Isolation Unit Model 305-1 (WPI, Hamden, CT, USA) at a frequency of 1 Hz and a pulse duration of 3 ms. Amplitude of stimulation pulse was adjusted 10% above threshold level. Signals were recorded with a dual beam storage oscilloscope Type 5113 (Tektronix Inc., Beaverton, Oregon, USA) and a chart recorder (BD 112 Dual Channel, Kipp & Zonen). Photos were taken every five minutes (Grass Camera Model C 45, Grass Instr. Co., Quincy, Massachusetts, USA), and were evaluated after magnification.

For statistical analyses the arithmetic means and standard error of the mean (SEM) of n experiments were calculated. Statistical significance of the results was evaluated by the Student's t-test for paired observations.

Stock solutions of the fumarate compound MS 63, the hydrochloride compound MS 65 and the oxalate compounds MS 64, MS 66 and MS 84 (Institute of Pharmaceutical Chemistry, University of Vienna) were prepared in distilled water every day and were further diluted with Krebs-Henseleit solution to the required concentrations.

To study the inotropic and chronotropic activity, after a control period of 30 min different concentrations of the compounds were added to the bathing solution cumulatively, until a steady state was reached.

4.2. Electromechanical studies on aortic strips

After excision of the heart, the aorta was dissected and stored at room temperature in gassed (95% O_2 – 5% CO_2), modified Krebs-Henseleit solution with the following composition (in mmol/l): NaCl 118.0, KCl 4.8, $CaCl_2$ 1.8, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24.0 and glucose 11. The aorta was cleaned of loosely adhering fat and connective tissue and helically cut into strips of 2 cm length and 4 mm width. The aortic strip was placed in a continuously oxygenated (95% O_2 and 5% CO_2) bath of 35 ml nutrient solution at a temperature of 37 ± 1 °C with one end connected to a tissue holder and the other to a force transducer and amplifier (Transbridge™, 4-Channel Transducer Amplifier, World Precision Instruments, Sarasota, FL, USA) for measurement of isometric contractions. Isometric tension was recorded with a dual beam storage oscilloscope Type 5113 (Tektronix Inc., Beaverton, Oregon, USA) and a chart recorder (BD 112 Dual Channel, Kipp & Zonen). Resting tension of 19.6 mN was kept constant throughout the experiments. Aortic strips were stimulated with 90 mmol/l KCl. Stock solutions of the compounds were prepared in distilled water every day and were further diluted with Krebs-Henseleit solution to the required concentrations.

To study the relaxing effects, after a control period of 60 to 120 min with 90 mmol/l KCl different concentrations of the compounds were added to the bathing solution cumulatively, until a steady state was reached.

4.3. Electromechanical studies on terminal ilea

The terminal portion of the ileum was removed and the 10 cm nearest to the caecum was discarded. The intestine was placed in a nutrient solution containing (in mmol/l): NaCl 136.90, KCl 2.7, $CaCl_2$ 1.80, $MgCl_2$ 1.05, NaH_2CO_3 24.0, NaH_2PO_4 0.43 and glucose 11. The intestine was cleaned by flushing with nutrient solution, cut into 2–3 cm long pieces and placed in a continuously oxygenated (95% O_2 and 5% CO_2) bath of 35 ml nutrient solution at a temperature of 35 ± 1 °C with one end connected to a tissue holder and the other to a force transducer and amplifier (Transbridge™, 4-Channel Transducer Amplifier, World Precision Instruments, Sarasota, FL, USA) for measurement of isometric contractions. Isometric tension was recorded with a dual beam storage oscilloscope Type 5113 (Tektronix Inc., Beaverton, Oregon, USA) and a chart recorder (BD 112 Dual Channel, Kipp & Zonen). Resting tension of 4.9 mN was kept constant throughout the experiments. Ilea were stimulated with 60 mmol/l KCl. Stock solutions of the compounds were prepared in distilled water every day and were further diluted with nutrient solution to the required concentrations. To study the relaxing effects, after a control period of 60 min with 60 mmol/l KCl different concentrations of the compounds were added to the bathing solution cumulatively, until a steady state was reached.

Acknowledgements: We thank Pakiza Saleh for her excellent technical assistance; M. Schreder and T. Erker for providing the compounds. This work was also supported by a grant for "Effects of new calcium antagonistic drugs and potassium channel openers on heart muscle preparations" by the University Foundation of the City of Vienna.

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Received August 18, 1998
Accepted October 10, 1998

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