Comparative Studies of New Thienothiazine Derivatives on Heart and Smooth Muscle Preparations of Guinea Pigs

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New thienothiazine derivatives which differ in their side chain on the nitrogen atom of the thienothiazine molecule were studied in guinea pig papillary muscles and terminal ileum using isometric contraction force measurements. Compounds with a heterocyclic ring in their side chain like MS 57 (pyrrolidinylthiocolboxamide side chain), MS 58 (piperidinoethylcarboxamide side chain) and MS 55 (morpholinoethylcarboxamide side chain) had the most potent negative inotropic effect on isolated papillary muscles. The relaxing effect on smooth muscle was more pronounced with compounds carrying an aromatic ring in their side chain like MS 25 (dimethoxyphenylethyl-N-aminopropionyl side chain), MS 24 (dimethoxyphenylethyl-N-methylaminocetyl side chain) and MS 27 (dimethoxyphenyl-N-methylaminocetylcarboxamide side chain). Our results show a tissue selectivity of the thienothiazine compounds.

Key words thienothiazine derivative; papillary muscle; terminal ileum; contraction force; calcium antagonist

New thienothiazine derivatives were studied on guinea pig heart and smooth muscle preparations. The compounds differ in their substituents on the N-atom of the thienothiazine molecule resulting in the compounds N-dimethylaminomethyl-6-ethyl-2,3-dihydro-1H-thieno[2,3-b][1,4]thiazine-1-carboxamide (MS 23), 1-[N-[(3,4-dimethoxyphenyl)cyclohexyl]-N-methylaminocetyl]-6-ethyl-2,3-dihydro-1H-thieno[2,3-b][1,4]thiazine (MS 24), 6-ethyl-2,3-dihydro-N-[(3,4-dimethoxyphenyl)cyclohexyl]-N-methylaminocetyl]-1H-thieno[2,3-b][1,4]thiazine (MS 25), N-[(3,4-dimethoxyphenyl)cyclohexyl]-N-methylaminocetyl]-6-ethyl-2,3-dihydro-1H-thieno[2,3-b][1,4]thiazine-1-carboxamide (MS 27), 6-ethyl-2,3-dihydro-N-(2-(4-morpholino)ethyl)-1H-thieno[2,3-b][1,4]thiazine carboxamide (MS 55), 6-ethyl-2,3-dihydro-N-(2-(1-pyrrolidinyl)ethyl)-1H-thieno[2,3-b][1,4]thiazine carboxamide (MS 57), 6-ethyl-2,3-dihydro-N-(2-(4-piperidino)ethyl)-1H-thieno[2,3-b][1,4]thiazine carboxamide (MS 58) and N-[2-(6,7-dimethoxy-1,2,3,4-tetrahydro-2-isocholino)ethyl]-6-ethyl-2,3-dihydro-1H-thieno[2,3-b][1,4]thiazine-1-carboxamide (MS 68) (Fig. 1).

These substances also have structural similarities with calcium antagonists like diltiazem or KT-362. Diltiazem and KT-362 show a negative inotropic and chronotropic activity on isolated guinea pig heart muscle preparations. The aim of this investigation was to study the structure-activity relationships of these drugs and to compare them to those of calcium antagonistic drugs. Thus the inotropic, chronotropic and relaxing effect of MS 23, MS 24, MS 25, MS 27, MS 55, MS 57, MS 58 and MS 68 was studied on isolated papillary muscles and terminal ilea of guinea pigs.

MATERIALS AND METHODS

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. The preparations were isolated and stored at room temperature in gassed (95% O2-5% CO2) Krebs–Henseleit solution with the following composition (in mmol/l): NaCl 114.9, KCl 4.73, CaCl2 3.2, MgSO4 1.18, NaHCO3 24.9, KH2PO4 1.18, glucose 10; pH 7.2—7.4. Isometric contraction force of electrically stimulated papillary muscles was measured by the method described by Reiter.24 Experiments were performed at a temperature of 35 ± 1°C. The bathing solution was continuously bubbled by a mixture of 95% O2 and 5% CO2 to guarantee sufficient oxygen supply and appropriate pH as well as circulation of nutrient solu-

![Fig. 1. Chemical Structures of the Thienothiazine Derivatives](image_url)
tion with and without test substance. A force transducer and amplifier (Transbridge™, 4-Channel Transducer Amplifier, World Precision Instruments, Sarasota, FL, U.S.A.) was used for measurement of isometric contractions. Resting tension of 3.92 mN was kept constant throughout the experiments.

Papillary muscles were electrically driven with an Ana

pulse Stimulator Model 301-T and an Isolation Unit Model 305-1 (WPI, Hamden, CT, U.S.A.) 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, U.S.A.) and a chart recorder (BD 112 Dual Channel, Kipp & Zonen). Photos were taken every 5 min (Grass Camera Model C 45, Grass Instr. Co., Quincy, MA, U.S.A.), and were evaluated after magnification. Stock solutions of the oxalate compounds MS 23, MS 24, MS 25, MS 27, MS 55, MS 57 and MS 58, and the hydrochloride compound MS 68 (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 activity, after a control period of 30 min the different concentrations of compounds were added to the bathing solution cumulatively, until a steady state was reached.

To study the Ca²⁺ antagonistic effect, the compounds MS 23, MS 24, MS 25, MS 27, MS 55, MS 57 and MS 58 were added in different concentrations to papillary muscles driven at 1 Hz. When a steady state was reached, CaCl₂ was added in increasing concentrations to antagonize the negative inotropic effect caused by the compounds.

**Electromechanical Studies on Terminal Ileum** 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, CaCl₂ 1.80, MgCl₂ 1.05, NaH₂CO₃ 24.0, NaHPO₄ 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% O₂ and 5% CO₂) 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 (Transbridge™, 4-Channel Transducer Amplifier, World Precision Instruments, Sarasota, FL, U.S.A.). Isometric tension was recorded with a dual beam storage oscilloscope Type 5113 (Tektronix Inc., Beaverton, Oregon, U.S.A.) and a chart recorder (BD 112 Dual Channel, Kipp & Zonen). Resting tension of 4.9 mN was kept constant throughout the experiments. Ileae were precontracted with 60 mmol/l KCl. Stock solutions of the compounds were prepared in distilled water daily 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 the different concentrations of compounds were added to the bathing solution cumulatively, until a steady state was reached.

**Statistics** 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.

### RESULTS

**Effects on Papillary Muscles and Terminal Ileum** The inotropic effect of the compounds was studied on isolated papillary muscles at a constant stimulation frequency of 1 Hz. MS 23 (1—100 μmol/l, n=6) concentration-dependently decreased force of contraction (fₒ) in a stimulation period (t₁) and time to peak force (t₂) were not significantly changed, but maximum rate of force development (sₓmax) and maximum rate of force relaxation (sｚmax) were significantly reduced. MS 24 in a concentration range between 1 and 100 μmol/l (n=6) concentration-dependently reduced fₒ, t₁, t₂ were not significantly changed, sₓmax and sｚmax were significantly decreased in a concentration-dependent manner. MS 25 (1—100 μmol/l, n=5) concentration-dependently decreased fₒ, sₓmax and sｚmax, whereas t₁ and t₂ were not significantly reduced. A concentration-dependent decrease of fₒ was also caused by MS 27 (0.3—100 μmol/l, n=7), MS 55 (0.3—100 μmol/l, n=4), MS 57 (0.03—100 μmol/l, n=4), MS 58 (0.03—30 μmol/l, n=4) and MS 68 (1—300 μmol/l, n=4). MS 27, MS 55, MS 57, MS 58 and MS 68 significantly reduced the other parameters of the contraction curve (t₁, t₂, sₓmax, sｚmax). The EC₅₀ for the compounds is presented in Table 1 and changes in t₁, t₂, sₓmax and sｚmax, are shown in Table 2. The decrease in percent of fₒ of the compounds is shown in Fig. 2.
The negative inotropic effect of the thienothiazine derivatives could be antagonized by CaCl₂. The negative inotropic effect of the EC₃₀, EC₅₀ and EC₇₀ of the compounds was antagonized by CaCl₂ at concentrations between 4.4 and 9.6 mmol/l (n=3--4). Table 3 shows the molecular ratio of the antagonism for the EC₃₀, EC₅₀ and EC₇₀ of the compounds and the concentration of CaCl₂.

Terminal ilea were precontracted with 60 mmol/l KCl. The relaxing effect of the compounds on contraction was studied in concentrations between 0.03 and 100 μmol/l. Again, all compounds concentration-dependently relaxed the 60 mmol/l KCl-induced contraction. The graphically estimated EC₉₀ values are presented in Table 1 and the decrease in percent of fC of the compounds is shown in Fig. 3.

**DISCUSSION**

The eight derivatives differ in their side chains on the nitrogen atom of the thienothiazine molecule. MS 57 with a pyrrolidinylethylcarboxamide side chain had the most potent negative inotropic effect on isolated papillary muscles, followed by MS 58, which has a piperidinoethylcarboxamide side chain and MS 55, which has a morpholinylethylcarboxamide side chain. The negative inotropic effect of the compounds MS 23 with a dimethylaminolethylcarboxamide side chain, MS 25 with a dimethoxyphenylethyl-N-aminopropionyl side chain and MS 24 with a dimethoxyphenylethyl-N-methylaminoacetamide side chain was less pronounced.
MS 27 with a dimethoxyphenyl-N-methylethylaminoethylcarboxamide side chain and MS 68 with a dimethoxytetrahydroisocolinylethylcarboxamide side chain showed the weakest negative inotropic effect. The decrease in activity is as follows: MS 57>MS 58>MS 55>MS 25>MS 23>MS 24>MS 27>MS 68 with EC$_{50}$ ranging from 0.8 to 10 $\mu$mol/l. The EC$_{50}$ for the negative inotropic effect of the calcium antagonist diltiazem is 16.1 $\mu$mol/l$^{1}$ and the EC$_{50}$ for KT-362 is 7 $\mu$mol/l.$^{2}$ Compared to diltiazem all compounds had a more potent negative inotropic effect. The side chains of MS 24, MS 25 and MS 27 are very similar to that of KT-362 and the EC$_{50}$-values are comparable to the EC$_{50}$ of KT-362. The negative inotropic effect of diltiazem could be reversed by increasing the extracellular calcium concentration. The necessary calcium concentration to restore the original force of contraction was nearly proportional to the concentration of diltiazem, which means that the antagonism between diltiazem and CaCl$_2$ is apparently competitive.$^{1}$ The negative inotropic effect of the thienothiazine derivatives could also be antagonized by increasing concentrations of CaCl$_2$ but in a non-competitive manner. Therefore, it is concluded that these compounds do not have pure calcium antagonistic properties. Derivatives with a heterocyclic ring in their side chain have the strongest negative inotropic activity.

We also studied the effect of the thienothiazine derivatives on KCl precontracted terminal ilea. The most potent relaxing effect on terminal ilea showed MS 27, M 25, MS 24 and MS 68. The action of the other compounds was less pronounced. MS 23 had the weakest effect on contractile responses. The following relaxing potency was observed: MS 27>MS 25>MS 24>MS 68>MS 58>MS 57>MS 55>MS 23. 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.$^{3,6}$ KT-362 is an intracellular calcium inhibitor with antiarrhythmic and vasodilating effects.$^{7,8}$ In isolated arterial vessel rings KT-362 inhibits contractile responses to norepinephrine but not to increased extracellular potassium.$^{9}$ The vasodilatory effect therefore might be due to interference with the release of intracellular calcium.$^{9}$

Our results demonstrate a tissue selectivity of the compounds. Evaluation of the tissue selectivity by estimating the ratio of the EC$_{50}$ values of the derivatives in papillary muscles and terminal ilea resulted in a 51-fold, 19-fold and 17-fold stronger negative inotropic effect of MS 57, MS 58 and MS 55 than the relaxing effect, whereas the relaxing effect of MS 27, MS 25 and MS 24 on terminal ilea was 11-fold, 6-fold and 4-fold stronger than the negative inotropic effect.

We conclude that a heterocyclic ring in the side chain is responsible for the strong negative inotropic potency and that the aromatic ring in the side chain results in the most potent relaxing effect.

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**REFERENCES**