# Effects of Novel Synthesized Pyridothiazines on Various Guinea Pig Heart Muscle Preparations

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**Calcium channel blockers have become important tools in the treatment of cardiovascular disorders and other diseases. Hybridization of well established calcium antagonist subclasses was an attempt to optimize their pharmacological profile. The intension of this study was to investigate the electrophysiological properties of MM 10 and MM 11 two newly synthesized compounds structurally closely related to KT-362 (5-[3-[[2-(3,4-dimethoxyphenyl)ethyl]amino]-1-oxopropyl]-2,3,4,5-tetrahydro-1,5-benzothiazepine fumarate) in various isolated guinea pig heart muscle preparations by means of the conventional intracellular microelectrode technique. MM 10 (2,3-dihydro-1-[*N*-[2-(3,4-dimethoxyphenyl)ethyl]-*N*-methylaminoacetyl]-1*H*-pyrido[2,3-*b*][1,4]-thiazine fumarate) and MM 11 (2,3-dihydro-1-[*N*-[2-(3,4-dimethoxyphenyl)ethyl]-*N*-methylaminopropionyl]-1*H*-pyrido[2,3-*b*][1,4]thiazine fumarate) exerted very similar effects though the action of MM 11 was more pronounced. Whereas action potential amplitude and maximum upstroke velocity ($V_{max}$) in papillary muscle, left atria and spontaneously beating Purkinje fibers was not affected by the compounds in a concentration range from 3 to 30 $\mu$mol/l, action potential duration at 90% time to repolarization was significantly prolonged in a concentration-dependent manner. Action potential duration at 20% time to repolarization was decreased in spontaneously beating Purkinje fibers and remained unchanged in papillary muscles and left atria. In sinoatrial nodes both compounds reduced rate of activity, action potential amplitude, maximum upstroke velocity and slope of slow diastolic depolarization while time to repolarization was prolonged. In 3 out of 6 experiments with spontaneously beating Purkinje fibers, MM 11 (30 $\mu$mol/l) led to the occurrence of early afterdepolarizations with a take off potential between −50 and −60 mV. All observed effects were completely reversible during washout with drug-free physiological salt solution. From these results it was concluded that both compounds in addition to their calcium antagonistic properties might depress repolarizing potassium currents. In contrast to the mother compound KT-362 they do not seem to affect the fast sodium inward current. Replacement of the benzothiazepine nucleus by a pyridothiazine structure may weaken or even eliminate sodium channel blocking ability. Shortening of the side chain might result in a general loss in activity.**

**Key words** calcium channel antagonist; microelectrode technique; guinea pig heart muscle; KT-362 derivative; 1,4-pyridothiazine

Since the early work of Fleckenstein[1] calcium entry blockers have become important therapeutical tools. As a result of their ability to reduce the influx of calcium ions through voltage dependent channels substances like verapamil or diltiazem are effective in the treatment of essential hypertension and angina pectoris as well as they are useful therapeutics in migraine and cardiac arrhythmia.[2]

Hybridization of calcium antagonist subclasses like benzothiazepines and phenylalkylamines was an attempt to increase beneficial and/or minimize undesirable side effects. The compound KT-362 (5-[3-[[2-(3,4-dimethoxyphenyl)-ethyl]amino]-1-oxopropyl]-2,3,4,5-tetrahydro-1,5-benzothiazepine fumarate) represents such a hybrid containing structural elements of verapamil as well as of diltiazem (Fig. 1). Inter alia KT-362 was found to be effective in the suppression of digitalis and adrenaline induced arrhythmia,[3] to reduce ischemia-reperfusion injury in dogs[4] and to exert cardioprotective effects in the stunned canine myocardium.[5] As a result, KT-362 is currently undergoing clinical trials in Japan as an antiarrhytmic agent with additional clinical potential in treating myocardial ischemia and hypertension.[6] Thus it was of interest to investigate the electrophysiological properties of MM 10 (2,3-dihydro-1-[*N*-[2-(3,4-dimethoxyphenyl)ethyl]-*N*-methylaminoacetyl]-1*H*-pyrido[2,3-*b*][1,4]thiazine fumarate) and MM 11 (2,3-dihydro-1-[*N*-[2-(3,4-dimethoxyphenyl)ethyl]-*N*-methylaminopropionyl]-1*H*-pyrido[2,3-*b*][1,4]thiazine fumarate), two newly synthesized substances structurally closely related to KT-362 (Fig. 1) with regard to a possible structure–activity relationship.

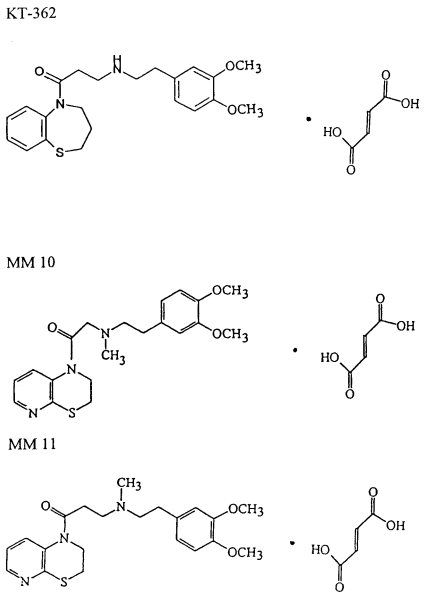


Fig. 1. Chemical Structures of KT-362, MM 10 and MM 11

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## MATERIALS AND METHODS

**Preparations** Guinea pigs of either sex (240—320 g) were killed by a blow on the neck and their hearts were removed rapidly *via* a midsternal thoracotomy. The left atrium was excised from the heart. Right atrium was further dissected. Only the area containing the sinoatrial node (approximate size of 0.5×0.5 cm) was used for experiments. Papillary muscles were isolated from the right ventricle after removing Purkinje fibers to prevent spontaneous activity. Purkinje fibers were carefully removed from the left ventricle, only spontaneously beating preparations were used for experiments.

**Experimental Setup** Preparations were mounted in a continuous-flow chamber. Papillary muscles and left atria were continuously stimulated with square wave impulses delivered by an anapulse stimulator and an isolation unit (World Precision Instruments, New Haven, CT, U.S.A.) at a frequency rate of 1 Hz. Membrane potentials and maximum upstroke velocities ($V_{max}$) were measured with respect to a grounded bath by using glass microelectrodes of 10—28 MΩ resistance filled with 3 M KCl and two amplifiers (M 701 and M 707 Microprobe System, World Precision Instruments, New Haven, CT, U.S.A.). Action potentials were recorded with a dual beam storage oscilloscope (Tektronix Inc., Beaverton, OR, U.S.A.). Photos of membrane potentials were taken every five minutes (Nihon Kohden Camera PC-2A, Nihon Kohden, Tokyo, Japan) and signals analyzed after magnification.

**Drugs and Solutions** During the experiments preparations were perfused with a modified Tyrode's solution containing (in mmol/l) NaCl 136.9, KCl 2.7 (for Purkinje fibers) or 5.4 (for left atria, papillary muscles and sinoatrial nodes), $MgCl_2$ 1.05, $NaH_2PO_4$ 0.42, $CaCl_2$ 1.8 and glucose 5.0. The solution was continuously bubbled with a mixture of 95% $O_2$ and 5% $CO_2$ at a temperature of 37±1 °C.

The investigated compounds MM 10 and MM 11 were synthesized at the Department of Pharmaceutical Chemistry, University of Vienna.[7] Drugs were dissolved in modified Tyrode's solution, stock solutions were prepared freshly every day.

**Experimental Protocols** After an equilibration period of at least one hour preparations were impaled and action potentials recorded for 30 min in drug-free Tyrode's solution. After this control period drugs were added at increasing concentrations until a steady state was reached followed by a washout period of 60 min. Continuous impalements were maintained throughout each experiment.

**Statistics** Data are presented as means±S.D. and evaluated statistically using Student's *t*-test for unpaired observations. A $p$ value less than 0.05 was considered to indicate a significant change.

**Chemical Syntheses** The syntheses of MM 10 and MM 11 is shown in Fig. 2. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. The $^1$H- and $^{13}$C-NMR spectra were recorded on a Varian UnityPlus 200 (200 MHz and 50 MHz). Chemical shifts are reported in $\delta$ values (ppm) relative to $Me_4Si$ line as internal standard and $J$ values are reported in Hertz. Mass spectra were obtained by a Shimadzu GC/MS QP 1000 EX or Hewlett Packard (GC: 5890; MS: 5970) spectrometer. The obtained elemental analysis results were within ±0.4% of the theoretical values for the formulas given. Solutions in organic solvents were dried over anhydrous sodium sulfate.

**2,3-Dihydro-1*H*-pyrido[2,3-*b*][1,4]thiazine (1)** Compound **1** was synthesized as reported by El-Subbagh *et al.*[7]

**Chloroacetyl-2,3-dihydro-1*H*-pyrido[2,3-*b*][1,4]thiazine (2)** To a solution of compound **1** (0.152 g, 1 mmol) in dry THF (5 ml) triethylamine (0.101 g, 1 mmol) and chloroacetyl chloride (0.170 g, 1.5 mmol) were added. The reaction mixture was stirred for 3 h at room temperature. After this the solvent was removed under reduced pressure. The residue was diluted with water and extracted three times with dichloromethane. The combined organic layers were dried over $Na_2SO_4$, filtered, and concentrated to give the crude product, which was purified by chromatography (toluene–ethylacetate, v/v=6 : 4) to give an oil. This was recrystallized from dil. ethanol to give **2** as pale yellow needles: mp, 87—90 °C; yield, 0.169 g, 74%. $^1$H-NMR ($CDCl_3$) $\delta$: 3.33 (2H, t, $J$=5.1 Hz, $CH_2$), 4.04 (2H, t, $J$=5.1 Hz, $CH_2$), 4.20 (2H, s, $CH_2Cl$), 7.08 (1H, dd, $J$=7.9 Hz, $J$=4.7 Hz, Ar-H), 7.63 (1H, s br, Ar-H), 8.30 (1H, d, $J$=4.7 Hz, Ar-H). $^{13}$C-NMR ($CDCl_3$) $\delta$: 28.2, 40.9, 119.1, 131.8, 132.6, 147.4, 165.4. MS *m*/*z*: 230/228 ($M^+$), 179, 153/151. *Anal.* Calcd for $C_9H_9ClN_2OS$: C, 47.27; H, 3.97; N, 12.25. Found: C, 47.06; H, 3.84; N, 11.98.

**1-[[*N*-[2-(3,4-Dimethoxyphenyl)ethyl]methylamino]-acetyl]-2,3-dihydro-1*H*-pyrido[2,3-*b*][1,4]thiazine (3)** To a solution of compound **2** (0.228 g, 1 mmol) in dry ethanol (8 ml) triethylamine (0.111 g, 1.1 mmol) and 2-(3,4-dimethoxyphenyl)-*N*-methyl-1-ethylamine (0.195 g, 1 mmol) were added. The reaction mixture was refluxed for 3 h. After this the solvent was removed under reduced pressure. The residue was diluted with water and extracted three times with dichloromethane. The combined organic layers were dried over $Na_2SO_4$, filtered, and concentrated to give a crude product, which was purified by chromatography (dichloromethane–methanol, v/v=20 : 1) to give an oil. Yield: 0.265 g, 68%. $^1$H-NMR ($CDCl_3$) $\delta$: 2.37 (3H, s, $NCH_3$), 2.69 (4H, s, $2CH_2$), 3.16—3.35 (4H, m, $2CH_2$), 3.84 (3H, s, $OCH_3$), 3.86 (3H, s, $OCH_3$), 3.91—3.99 (2H, m, $CH_2$), 6.68—6.81 (3H, m, Ar-H), 6.96 (1H, dd, $J$=8.1 Hz, $J$=4.7 Hz, Ar-H), 7.65 (1H, s br, Ar-H), 8.25 (1H, d, $J$=4.7 Hz, Ar-H). $^{13}$C-NMR ($CDCl_3$) $\delta$: 28.6, 31.8, 33.1, 41.7, 53.2, 55.5, 55.6, 59. 5, 61.4, 111.0, 111.7, 118.7, 125.0, 132.6, 132.7, 133.9, 137.5, 146.8, 147.0, 148.5, 171.1. MS *m*/*z*: 388 ($M^+$+1), 236, 165. *Anal.* Calcd for $C_{20}H_{25}N_3O_3S$: C, 61.99; H, 6.50; N, 10.84. Found: C, 61.70; H, 6.39; N, 10.71.

**1-[[*N*-[2-(3,4-Dimethoxyphenyl)ethyl]methylamino]-acetyl]-2,3-dihydro-1*H*-pyrido[2,3-*b*][1,4]thiazine Fumarate (MM 10)** To a solution of compound **3** (0.388 g, 1 mmol) and fumaric acid (0.116 g, 1 mmol) in dry ethanol (10 ml) dry ether was added gradually until a slight precipitation could be detected. This mixture was stored at 0 °C for 20 h. After this the precipitated solid was filtered to give MM 10 as white needles: mp, 119—121 °C; yield, 0.342 g, 68%. *Anal.* Calcd for $C_{24}H_{29}N_3O_7S$: C, 57.24; H, 5.80; N, 8.34. Found: C, 57.04; H, 5.65; N, 8.25.

**Acryloyl-2,3-dihydro-1*H*-pyrido[2,3-*b*][1,4]thiazine (4)** To a solution of compound **1** (0.152 g, 1 mmol) in dry THF (5 ml) triethylamine (0.101 g, 1 mmol) and chloropropionyl chloride (0.317 g, 2.5 mmol) were added. The reaction mix-

ture was stirred for 3 h at room temperature. After this the solvent was removed under reduced pressure. The residue was diluted with water and extracted three times with dichloromethane. The combined organic layers were dried over $Na_2SO_4$, filtered, and concentrated to give a crude product, which was purified by chromatography (dichloromethane–methanol, v/v=10 : 1) to give an oil. This was recrystallized from dil. ethanol to give **4** as white needles: mp, 137—139 °C; yield, 0.130 g, 63%. $^1$H-NMR ($CDCl_3$) $\delta$: 3.32 (2H, t, $J$=5.5 Hz, $CH_2$), 4.14 (2H, t, $J$=5.1 Hz, $CH_2$), 5.73—5.81 (1H, m, CH), 6.43—6.50 (2H, m, CH), 7.03 (1H, dd, $J$=8.0 Hz, $J$=4.7 Hz, Ar-H), 7.33 (1H, d, $J$=8.0 Hz, Ar-H), 8.29 (1H, dd, $J$=4.7 Hz, $J$=1.5 Hz, Ar-H). $^{13}$C-NMR ($CDCl_3$) $\delta$: 28.9, 40.1, 118.8, 127.9, 129.6, 132.7, 133.4, 146.9, 152.1, 164.4. MS *m/z*: 206 ($M^+$), 152, 137, 55. *Anal.* Calcd for $C_{10}H_{10}N_2OS$: C, 58.23; H, 4.89; N, 13.58. Found: C, 58.02; H, 4.76; N, 13.31.

**1-[3-[*N*-[2-(3,4-Dimethoxyphenyl)ethyl]methylamino]-propionyl]-2,3-dihydro-1*H*-pyrido[2,3-*b*][1,4]thiazine (5)** To a solution of compound **4** (0.206 g, 1 mmol) in dry ethanol (8 ml) 2-(3,4-dimethoxyphenyl)-*N*-methyl-1-ethylamine (0.195 g, 1 mmol) was added. The reaction mixture was refluxed for 3 h. After this the solvent was removed under reduced pressure. The residue was diluted with water and extracted three times with dichloromethane. The combined organic layers were dried over $Na_2SO_4$, filtered, and concentrated to give a crude product, which was purified by chromatography (toluene–ethylacetate–triethylamin, v/v =6 : 3 : 1) to give a pale yellow oil. Yield: 0.258 g, 64%. $^1$H-NMR ($CDCl_3$) $\delta$: 2.21 (3H, s, $NCH_3$), 2.52—2.79 (8H, m, $4CH_2$), 3.36 (2H, t, $J$=5.4 Hz, $CH_2$), 3.86 (3H, s, $OCH_3$), 3.87 (3H, s, $OCH_3$), 3.95—4.10 (2H, m, $CH_2$), 6.76 (1H, s, Ar-H), 6.69 (1H, d, $J$=7.8 Hz, Ar-H), 6.77 (1H, d, $J$=7.8 Hz, Ar-H), 7.01 (1H, dd, $J$=8.0 Hz, $J$=4.7 Hz, Ar-H), 7.41 (1H, s br, Ar-H), 8.28 (1H, dd, $J$=4.7 Hz, $J$=1.3 Hz, Ar-H). $^{13}$C-NMR ($CDCl_3$) $\delta$: 28.6, 31.9, 33.1, 41.7, 53.2, 55.5, 55.6, 59.5, 61.4, 110.9, 111.7, 118.7, 125.0, 132.6, 132.6, 133.9, 137.5, 146.8, 147.0, 148.5, 171.1. MS *m/z*: 402 ($M^+$), 250, 207. *Anal.* Calcd for $C_{21}H_{27}N_3O_3S$: C, 62.82; H, 6.78; N, 10.47. Found: C, 62.59; H, 6.75; N, 10.20.

**1-[3-[*N*-[2-(3,4-Dimethoxyphenyl)ethyl]methylamino]-propionyl]-2,3-dihydro-1*H*-pyrido[2,3-*b*][1,4]thiazine fumarate (MM 11)** To a solution of compound **5** (0.402 g, 1 mmol) and fumaric acid (0.116 g, 1 mmol) in dry ethanol (10 ml) dry ether was added gradually until a slight precipitation could be detected. This mixture was stored at 0 °C for 20 h. After this the precipitated solid was filtered to give MM 11 as white needles: mp, 62 °C; yield, 0.460 g, 89%. *Anal.* Calcd for $C_{25}H_{31}N_3O_7S$: C, 58.01; H, 6.04; N, 8.12. Found: C, 57.93; H, 5.89; N, 8.03.

## RESULTS

**Effects on Action Potential of Papillary Muscles** Application of MM 10 in concentrations of 3 $\mu$mol/l, 10 $\mu$mol/l and 30 $\mu$mol/l led to significant prolongation of action potential duration at 50% time to repolarization from 237.5±6.45 to 260.0±4.08 ms (3 $\mu$mol/l), to 267.5±2.98 ms (10 $\mu$mol/l) and to 268.75±4.79 ms (30 $\mu$mol/l) ($n$=5, $p$<0.05) as well as at 90% time to repolarization from 258.78±10.31 to 280.0±13.5 ms (3 $\mu$mol/l) ($n$=5, $p$<0.05), to 293.0±8.9 ms (10 $\mu$mol/l) and to 306.25±8.54 ms (30 $\mu$mol/l) ($n$=5, $p$<0.05), whereas no significant change in action potential duration at 20% time to repolarization ($APD_{20}$) could be observed. Membrane resting potential (MRP) as well as action potential amplitude (APA) and maximum rate of rise ($V_{max}$) were not affected in experiments with MM 10.

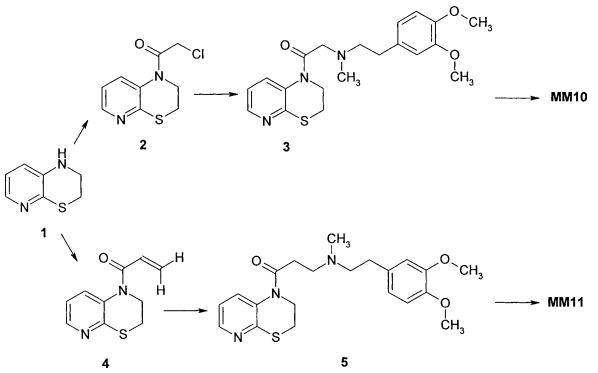


Fig. 2. Chemical Syntheses of MM 10 and MM 11

Used in the same concentrations MM 11 did not exert any effect on action potential amplitude, maximum upstroke velocity and membrane resting potential. Action potential duration at 50% time to repolarization was prolonged from 195.0±9.13 to 210.0±10.8 ms (3 $\mu$mol/l) and to 212.5±10.41 ms (30 $\mu$mol/l) ($n$=4, $p$<0.05) as well as at 90% time to repolarization from 212.25±4.79 to 246.25±14.36 ms (3 $\mu$mol/l) and to 262.5±9.57 ms (30 $\mu$mol/l) ($n$=4, $p$<0.05) prolonged in a concentration-dependent manner. All observed effects occurred immediately after drug application reached maximum in approximately 30 min and where completely reversible during washout with drug-free Tyrode's solution. Original recordings are shown in Fig. 2.

**Effects on Action Potential of Left Atria** Similar to experiments with papillary muscles action potential duration at 50% time to repolarization was clearly prolonged from 44.75±5.5 to 51.0±4.62 ms (3 $\mu$mol/l), to 58.0±5.72 ms (10 $\mu$mol/l) and to 66.75±7.68 ms (30 $\mu$mol/l) ($n$=4, $p$<0.05) as well as at 90% time to repolarization from 97.0±2.16 to 113.25±3.95 ms (3 $\mu$mol/l) ($n$=5, $p$<0.05), to 130.0±4.08 ms (10 $\mu$mol/l) and to 147.5±6.54 ms (30 $\mu$mol/l) ($n$=4, $p$<0.05), during exposure to MM 10. On the other hand the compound did not produce any change in $APD_{20}$, membrane resting potential, action potential amplitude or $V_{max}$.

Similar results could be obtained with MM 11 (3 $\mu$mol/l and 30 $\mu$mol/l). In these experiments action potential amplitude, $V_{max}$ and membrane resting potential remained unchanged, whereas the investigated substance produced significant prolongation in action potential duration at 50% time to repolarization was prolonged from 42.5±2.9 to 55.75±1.2 ms (3 $\mu$mol/l) and to 71.67±2.89 ms (30 $\mu$mol/l) ($n$=4, $p$<0.05) as well as at 90% time to repolarization from 96.25±9.46 to 123.75±8.54 ms (3 $\mu$mol/l) and to 161.67±10.41 ms (30 $\mu$mol/l) ($n$=4, $p$<0.05). In contrast to experiments with MM 10 $APD_{20}$ was as well prolonged from 21.25±2.5 to 28.75±2.5 ms (3 $\mu$mol/l) and to 33.33±2.89 ms (10 $\mu$mol/l) ($n$=4, $p$<0.05). Both substances started to exert their effects soon after application, maximum effects could be measured after approximately 30 min and were

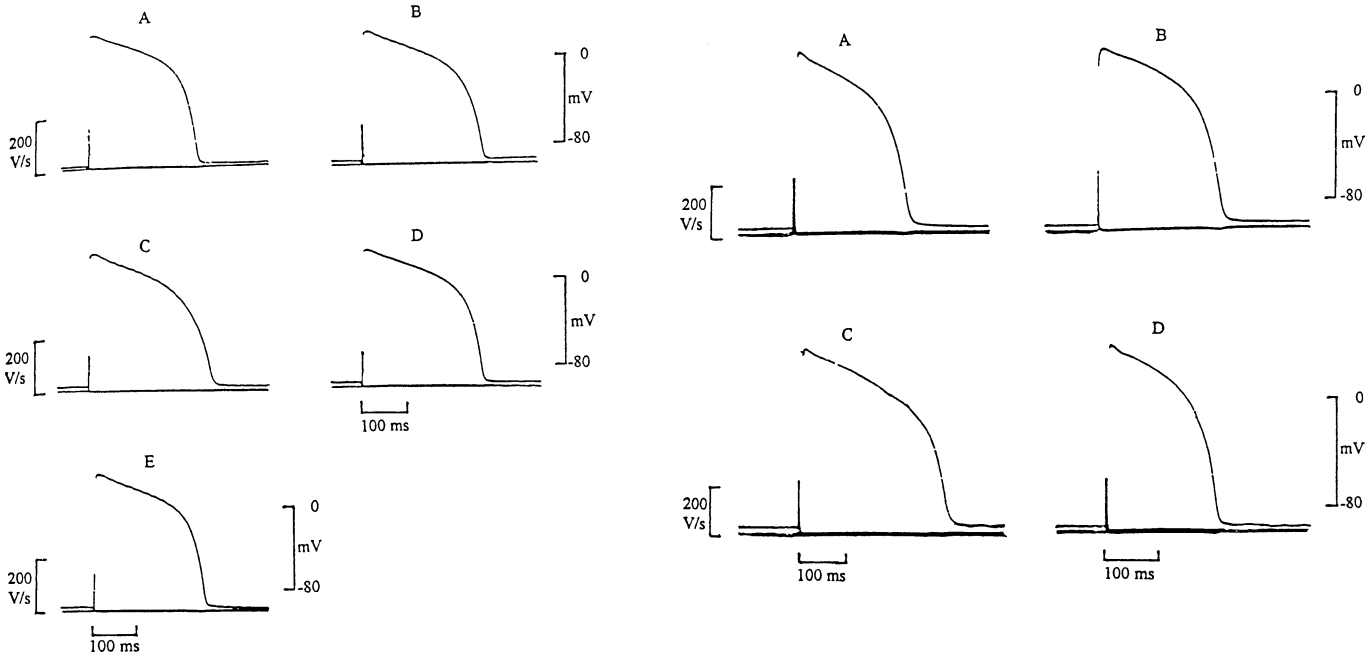


Fig. 3. Left Hand Panel: Transmembrane Action Potentials of a Guinea Pig Papillary Muscle in Control (A), after the Addition of 3 $\mu$mol/l MM 10 (B), after the Addition of 10 $\mu$mol/l MM 10 (C), after the Addition of 30 $\mu$mol/l MM 10 (D) and during Washout (E). Right Hand Panel: Transmembrane Action Potentials in Control (A), after the Addition of 3 $\mu$mol/l MM 11 (B), after the Addition of 30 $\mu$mol/l MM 11 (C) and during Washout (D)

The preparation was constantly driven at 1 Hz. Upper trace shows membrane action potential and lower trace the upstroke spike of the action potential ($V_{max}$).

completely reversible during washout. Figure 3 shows original recordings.

**Effects on Action Potential of Spontaneously Beating Purkinje Fibers** In spontaneously beating Purkinje fibers MM 10 led to a significant prolongation of $APD_{90}$ from 356.25±32.5 to 400.0±41.79 ms (3 $\mu$mol/l) and to 475.0±59.02 ms (10 $\mu$mol/l) ($n$=4, $p$<0.05). A concnetration of 30 $\mu$mol/l increased $APD_{90}$ 411.67±12.58 to 566.67±14.53 ($n$=4, $p$<0.05). In addition 30 $\mu$mol/l MM 10 produced a significant decrease in $APD_{20}$ from 130.0±17.32 to 90.0±17.32 ms as well as in slope of slow diastolic depolarization (SSDD) from 13.33±2.31 to 6.67±1.53 mV/s and rate of activity (RA) from 49.67±5.96 to 35.0±6.24 beats/min ($n$=4, $p$<0.05). Action potential amplitude, $V_{max}$ and maximum diastolic potential (MDP) were not affected by MM 10.

Exposure to MM 11 led to a concentration-dependent prolongation of action potential duration at 50% time to repolarization from 195.0±48.22 to 236.67±55.08 ms (3 $\mu$mol/l, $n$=3) and to 263.33±58.59 ms (30 $\mu$mol/l, $n$=3) as well as at 90% time to repolarization from 285.0±40.93 to 376.67±64.29 ms (3 $\mu$mol/l, $n$=3) and to 406.67±81.45 ms (30 $\mu$mol/l, $n$=3, $p$<0.05). Slope of slow diastolic depolarization and rate of activity were significantly decreased at a concentration of 30 $\mu$mol/l from 12.0±2.0 to 8.0±2.0 mV/s and ($n$=3, $p$<0.05) and from 44.0±4.58 to 33.0±3.0 beats/min ($n$=3, $p$<0.05). In 3 out of 6 experiments MM 11 in a concentration of 30 $\mu$mol/l led to the occurrence of early afterdepolarizations with a take-off potential between −50 and −60 mV in 4 to 6 min after drug application. Early afterdepolarizations and all other observed effects were completely reversible during washout. Original recordings from action potential and early afterdepolarizations are shown in Fig. 4.

**Effects on Action Potential of Sinoatrial Nodes** In a concentration of 30 $\mu$mol/l MM 10 significantly reduced rate of activity from 186.0±24.0 to 140.0±33.05 beats/min ($n$=3, $p$<0.05) and slope of slow diastolic depolarization from 69.0±3.61 to 41.67±7.64 mV/s ($n$=3, $p$<0.05) in sinoatrial nodes. Maximum diastolic potential was decreased from −59.6±1.77 to −53.7±2.05 mV ($n$=3, $p$<0.05) as well as action potential amplitude from 73.33±2.89 to 61.67±5.77 mV ($n$=3, $p$<0.05) and $V_{max}$ from 16.67±5.77 to 10.0±4.36 V/s ($n$=3, $p$<0.05). On the other hand the compound produced significant prolongation of 50% time to repolarization from 98.33±2.89 to 123.33±5.77 ms ($n$=3, $p$<0.05) and 90% time to repolarization from 132.0±8.19 to 181.67±10.41 ms ($n$=3, $p$<0.05) whereas 20% time to repolarization was not affected.

In the same concentration MM 11 exerted similar but more pronounced effects. In a concentration of 30 $\mu$mol/l MM 11 significantly reduced rate of activity from 192.0±0 to 114.0±15.87 beats/min ($n$=3, $p$<0.05) and slope of slow diastolic depolarization from 62.67±2.52 to 38.33±2.89 mV/s ($n$=3, $p$<0.05) in sinoatrial nodes. Maximum diastolic potential was decreased from −60.0±0 to −48.33±2.31 mV ($n$=3, $p$>0.05) as well as action potential amplitude from 70.33±2.89 to 52.33±4.62 mV ($n$=3, $p$<0.05) and $V_{max}$ from 20.67±1.15 to 8.0±2.0 V/s ($n$=3, $p$<0.05). 50% time to repolarization increased from 90.0±0 to 112.0±3.46 ms ($n$=3, $p$<0.05) and 90% time to repolarization from 118.33±2.89 to 185.33±0.58 ms ($n$=3, $p$<0.05) whereas 20% time to repolarization was not affected.

In the same concentration MM 11 exerted similar but more pronounced effects. In these experiments effects as well occurred soon after drug application reached maximum in approximately 30 min and were completely reversible during washout with drug-free physiological salt solution.

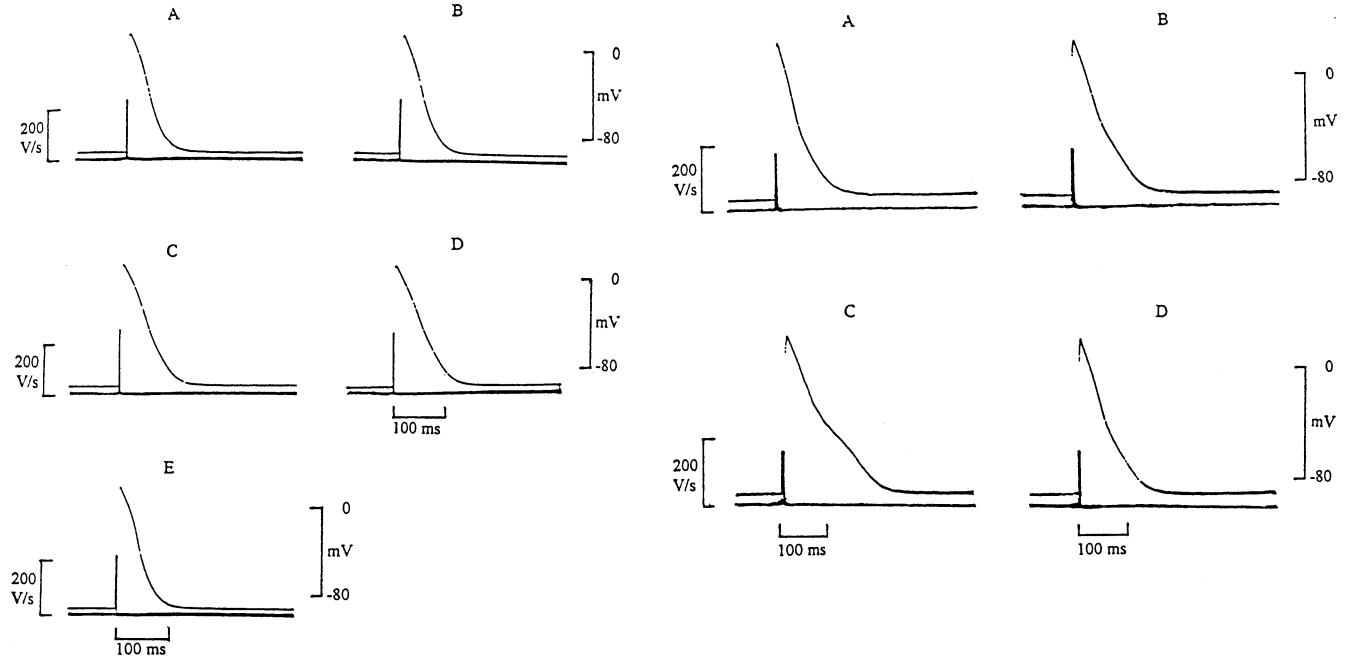


Fig. 4. Left Hand Panel: Transmembrane Action Potentials of a Left Atrium in Control (A), after the Addition of 3 $\mu$mol/l MM 10 (B), after the Addition of 10 $\mu$mol/l MM 10 (C), after the Addition of 30 $\mu$mol/l MM 10 (D) and during Washout (E). Right Hand Panel: Transmembrane Action Potentials in Control (A), after the Addition of 3 $\mu$mol/l MM 11 (B), after the Addition of 30 $\mu$mol/l MM 11 (C) and during Washout (D)

The preparation was constantly driven at 1 Hz. Upper trace shows membrane action potential and lower trace the upstroke spike of the action potential ($V_{max}$).

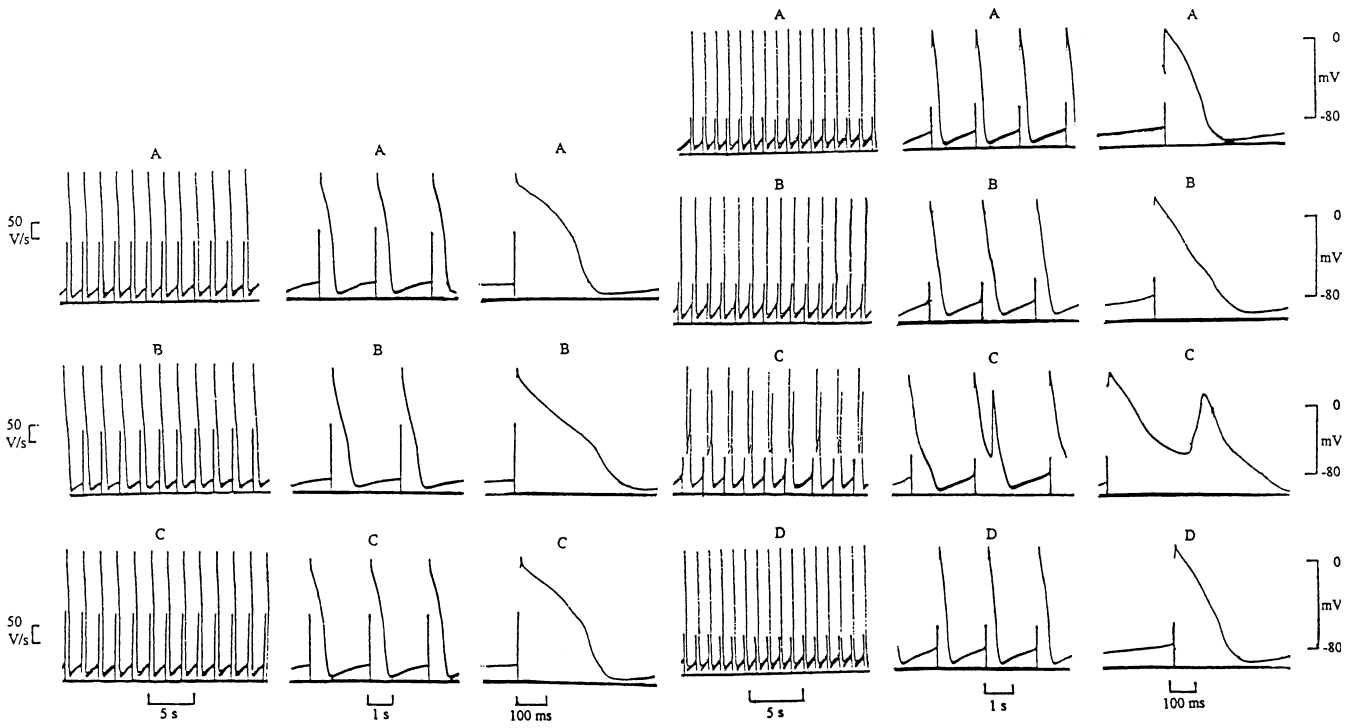


Fig. 5. Left Hand Panel: Transmembrane Action Potentials of a Guinea Pig Spontaneously Beating Purkinje Fibre in Control (A), after the Addition of 30 $\mu$mol/l MM 10 (B) and during Washout (C). Right Hand Panel: Transmembrane Action Potentials in Control (A), after the Addition of 3 $\mu$mol/l MM 11 (B), after the Addition of 30 $\mu$mol/l MM 11 (C) and during Washout (D)

Upper trace shows membrane action potential and lower trace the upstroke spike of the action potential ($V_{max}$).

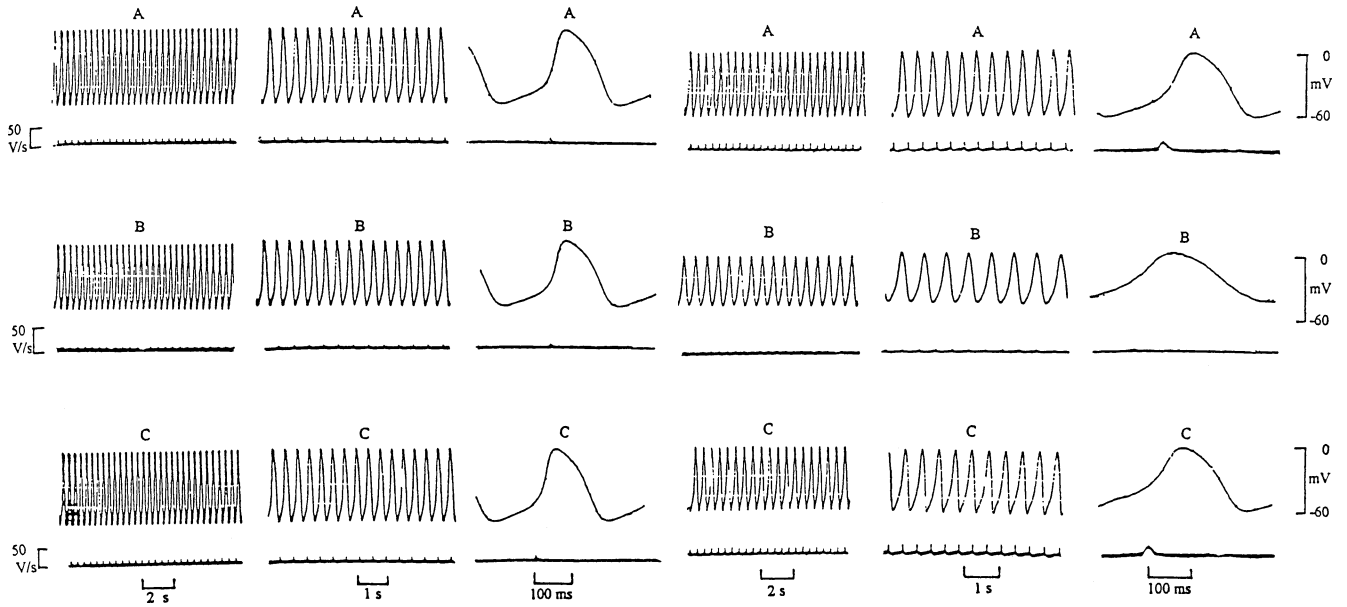


Fig. 6. Left Hand Panel: Transmembrane Action Potentials of a Guinea Pig Sinoatrial Node in Control (A), after the Addition of 30 $\mu$mol/l MM 10 (B) and during Washout (C). Right Hand Panel: Transmembrane Action Potentials in Control (A), after the Addition of 30 $\mu$mol/l MM 11 (B) and during Washout (C)

Upper trace shows membrane action potential and lower trace the upstroke spike of the action potential ($V_{max}$).

## DISCUSSION

Although the effects of MM 11 were more pronounced both compounds exerted very similar electrophysiological profiles. Since phase 0 of action potential of papillary muscles, left atria and Purkinje fibers is generated by fast influx of sodium ions, action potential amplitude and $V_{max}$ are good indicators of fast inward current $I_{Na}$.[8] This current seems not to be affected by the investigated substances considering the fact that neither MM 10 nor MM 11 produced any change in APA or $V_{max}$ of these preparations. While the action potential duration is maintained by a balance between influx of calcium ions ($I_{si}$) during the plateau phase and the activation of several repolarizing outward potassium currents like $I_K$ and/or $I_{K1}$.[9,10] So prolongation of action potential duration as it was caused by MM 10 and MM 11 in papillary muscles and left atria as well as in Purkinje fibers may be due to either an increase in $I_{si}$ or a decrease in outward current. The first possibility is rather unlikely for both compounds exerted negative inotropic effects in isolated guinea pig papillary muscles.[11] Depression of potassium currents and concomitant prolongation of action potential duration at 90% time to repolarization may as well provide an explanation for the decrease in rate of activity that could be observed in experiments with Purkinje fibers. Additionally, MM 11 was found to depress slope of slow diastolic depolarization in these preparations, probably due to a decrease in pacemaker current.

The fact that action potential duration at 20% and 50% time to repolarization was shortened or not affected by MM 10 and MM 11 can be attributed to a decrease in $I_{si}$ in part overlapped by the simultaneous delay in repolarization. This assumption is supported by the results obtained in experiments with sinoatrial nodes. In this preparation phase 0 and phase 1 of transmembrane action potential are mainly dependent on depolarizing slow inward calcium current whereas activation of fast sodium channels does not seem to play any role.[12,13] Application of MM 10 and MM 11 led to significant decreases in action potential amplitude and $V_{max}$ as well as in slope of slow diastolic depolarization and rate of activity, as it is expected for calcium channel antagonists.

Though experimental data from these experiments do not provide direct evidence concerning the influence of MM 10 and MM 11 on depolarizing and/or repolarizing currents, following conclusions may be reasonable: (i) fast sodium current seems not to be affected, (ii) $I_{si}$ is decreased as well as (iii) one or more repolarizing potassium currents. According to the model of January and Riddle[14,15] the delay in repolarization caused by MM 11 may provide for the "conditioning phase" preceeding the activation of a depolarizing inward current and finally leading to the occurrence of early afterdepolarizations.

In contrast to its derivatives KT-362 led to a concentration-dependent reduction in action potential amplitude and $V_{max}$ in guinea pig ventricular muscle.[16] In isolated guinea pig hearts KT-362 produced a concentration-dependent decrease in atrial rate with an $EC_{50}$ of 20 $\mu$mol/l.[17] In addition to an inhibition of the L-type calcium current Yuan-Na and coworkers[18] found a decrease in peak sodium current in guinea pig ventricular myocytes in a concentration range from 10 to 30 $\mu$mol/l. In the same study KT-362 inhibited the delayed rectifier potassium current and the inward rectifier potassium current. The compound is as well considered to act in a ryanodine-like manner as an inhibitor of intracellular calcium release.[19,20] Concerning the structure–activity relationship the results of this study gave rise to the assumption, that the replacement of the benzothiazepine nucleus by a pyridothiazine ringsystem leads to a weakening or even a loss of sodium channel blocking ability whereas calcium antagonistic characteristics as well as depressing effects on potassium currents are preserved. Shortening of the side chain may account for the fact that the effects exerted by MM 11, particu-

larly those on repolarization, were more pronounced than that performed by MM 10. Whether the investigated 1,4-pyridothiazines in accordance with the mother compound KT-362 act on intracellular calcium stores and which ion currents they affect in particular is left to further investigations.

## REFERENCES


1) Fleckenstein A., *Annu. Rev. Pharmacol. Toxicol.*, **17**, 149—166 (1977).
2) Braunwald E., *New Engl. J. Med.*, **26**, 1618—1627 (1982).
3) Hashimoto K., Watanabe K., Mochizuki S., Tomiyama A., *Jpn. J. Pharmacol.*, **51**, 475—482 (1989).
4) Pelc L. R., Farber N. E., Warltier D. C., Gross G. J., *J. Cardiovasc. Pharmacol.*, **13**, 586—593 (1989).
5) Farber N. E., Gross G. J., *J. Pharmacol. Exp. Ther.*, **248**, 39—43 (1989).
6) Hester R. K., Shibata S., *Cardiovasc. Drugs Ther.*, **4**, 1345—1354 (1990).
7) El-Subbagh H. I., Abadi A. H., Al-Khawad I. E., Al-Rashood K. A., *Arch. Pharm. Pharm. Med. Chem.*, **332**, 19—24 (1999).
8) Weidmann S., *J. Physiol.* (London), **127**, 213 (1955).
9) Sakmann B., Trube G., *J. Physiol.* (London), **347**, 641—657 (1984).
10) Sanguinetti M. C., Jurkiewicz N. K., *J. Gen. Physiol.*, **96**, 195—215 (1990).
11) Schade B., Studenik C., *Biol. Pharm. Bull.*, **22**, 683—686 (1999).
12) Yamagishi S., Sano T., *Proc. Jpn. Acad.*, **42**, 1194—1196 (1966).
13) Kreitner D., *J. Mol. Cell. Cardio.*, **7**, 655—662 (1975).
14) January C. T., Riddle J. M., *Cir. Res.*, **64**, 977—990 (1989).
15) January C. T., Riddle J. M., Salata J. J., *Circ. Res.*, **62**, 563—571 (1988).
16) Kodama I., Wakabayashi S., Toyama J., Shibata S., Yamada K., *J. Cardiovasc. Pharmacol.*, **11**, 687—693 (1988).
17) Buljubasic N., Marijic J., Stowe D. F., Gross G. J., Kampine J. P., Bosnjak Z. J., *J. Cardiovasc. Pharmacol.*, **18**, 594—604 (1991).
18) Yuan-Na C., Tatsuko K., Makoto A., *J. Pharmacol. Exp. Ther.*, **270**, 851—857 (1994).
19) Kodama I, Shibata S., *J. Pharmacol. Exp. Ther.*, **258**, 332—338 (1991).
20) Eskinder H., Hillard C. J., Wilke R. A., Gross G. J., *J. Cardiovasc. Pharmacol.*, **13**, 502—507 (1989).