Inhibition of cardiac Kv1.5 potassium current by the anesthetic midazolam: mode of action

Nadine Vonderlin¹
Fathima Fischer¹
Edgar Zitron^{1,2}
Claudia Seyler¹
Daniel Scherer¹
Dierk Thomas^{1,2}
Hugo A Katus^{1,2}
Eberhard P Scholz¹

¹Department of Internal Medicine III, University Hospital Heidelberg, Heidelberg, Germany;
²German Centre for Cardiovascular Research (DZHK), Partner Site Heidelberg/Mannheim, Heidelberg, Germany

Abstract: Midazolam is a short-acting benzodiazepine that is widely used in anesthesia. Despite its widespread clinical use, detailed information about cardiac side effects of midazolam is largely lacking. Using the double-electrode voltage clamp technique, we studied pharmacological effects of midazolam on heterologously expressed Kv1.5 channels underlying atrial repolarizing current I_{Kur} . Midazolam dose-dependently inhibited Kv1.5 current, yielding an IC_{50} of 17 μ M in an HEK cell line and an IC_{50} of 104 μ M in *Xenopus* oocytes. We further showed that midazolam did not affect the half-maximal activation voltage of Kv1.5 channels. However, a small negative shift of the inactivation curve could be observed. Midazolam acted as a typical open-channel inhibitor with rapid onset of block and without frequency dependence of block. Taken together, midazolam is an open channel inhibitor of cardiac Kv1.5 channels. These data add to the current understanding of the pharmacological profile of midazolam.

Keywords: anesthetics, potassium channels, pharmacology

Introduction

Midazolam is a short-acting imidazobenzodiazepine that is widely used in anesthesia.^{1,2} Similar to classic benzodiazepines, midazolam potentiates the effect of GABA by increasing the frequency of chloride channel opening in the brain.^{3,4} From a clinical point of view, midazolam bears a number of beneficial characteristics, such as a rapid onset of action as well as the possibility of pharmacological antagonism.¹ Considering its favorable pharmacological profile, midazolam is increasingly used by cardiologists in patients undergoing electrophysiological procedures such as electrical cardioversion, catheter ablation of atrial fibrillation, or catheter ablation of ventricular tachycardia.^{5–7} Within this context, detailed information on the pharmacological side effects of midazolam on cardiac electrophysiology seems necessary. Interestingly, a variety of other anesthetics have been shown to interfere with cardiac ion channels, thereby exerting potential pro- or antiarrhythmic side effects (for review, see Hüneke et al⁸ and Staikou et al⁹). For example, Miró et al published a small case series of patients exhibiting a conversion from atrial fibrillation to sinus rhythm after intravenous administration of propofol.¹⁰ From the literature available so far, midazolam has not been associated with severe pro- or antiarrhythmic side effects. However, it is well recognized that midazolam application might influence cardiac repolarization, thereby prolonging the QT interval.^{9,11} In experimental studies, midazolam has been shown to exert inhibitory effects on the calcium-independent transient outward current I_{to} and the inward rectifier current I_{K1} , as well as on a neural delayed rectifier current $I_{K(DR)}$.^{12,13} However, effects of midazolam on the main repolarizing atrial delayed rectifier current I_{Kur} or its molecular correlate, the Kv1.5 ion channel, have not yet been reported.

Correspondence: Eberhard P Scholz
Department of Internal Medicine III
(Cardiology), University Hospital
Heidelberg, Im Neuenheimer Feld 410,
69120 Heidelberg, Germany
Tel +49 6221 56 8611
Fax +49 6221 563 3497
Email eberhard.scholz@med.uni-heidelberg.de

The family of voltage-gated K⁺ channels (Kv) is characterized by a pore-forming α -subunit containing six membrane-spanning domains, a highly charged voltage-sensing S4 domain, and a K⁺ selectivity filter.^{1,14} Kv channels play an essential role in the regulation of the cardiac action potential. As a consequence, pharmacological inhibition of Kv channels represents a cornerstone of antiarrhythmic drug therapy. Among the family of Kv channels, Kv1.5 belongs to the first cloned Kv subfamily, termed “Shaker”. When heterologously expressed, Kv1.5 channels give rise to ultra-rapidly activating and merely inactivating potassium current. Kv1.5 has been identified as the molecular basis of the repolarizing ultra-rapidly activating delayed rectifier current I_{Kur} in human atrial myocytes.^{15,16} Hence, inhibition of I_{Kur} results in action potential prolongation in isolated trabeculae from human atria.¹⁷ Nearly absent in the ventricles and highly expressed in human atria, Kv1.5 represents a promising pharmacological target for atrial selective antiarrhythmic therapy.¹⁸ As a consequence, much effort is being devoted to identifying small molecule-inhibitors of Kv1.5.

Pharmacological inhibition of cardiac ion channels is a well-recognized side effect that has been reported for a number of clinically established anesthetics.^{19,20} However, despite its widespread clinical use, the effects of midazolam on cardiac ion channels have not yet been systematically studied. So far, inhibitory effects of midazolam on the calcium-independent transient outward current I_{to} , the inward rectifier potassium current I_{K1} , and a neural delayed rectifier current ($I_{K(DR)}$) have been described.^{12,13} The aim of the present study was to characterize pharmacological effects of midazolam on cardiac Kv1.5 channels underlying the important atrial repolarizing potassium current I_{Kur} . These data might, on the one hand, provide better insights into the pharmacological profile of midazolam and, on the other hand, help identify new lead compounds for the development of Kv1.5 inhibitors.

Methods

Drugs, chemicals, and drug administration

Midazolam (Hoffman-La Roche Ltd., Basel, Switzerland) was stored and handled according to the manufacturer's specifications. On the day of the experiment, the stock solution was further diluted to the desired concentration with external solution. External and internal solutions were stored at 4°C. Double-electrode voltage-clamp experiments were carried out using a standard external solution containing 5 mM KCl, 100 mM NaCl, 1.5 mM CaCl₂, 2 mM MgCl₂, and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic

acid (HEPES) (pH adjusted to 7.4 with NaOH). Electrodes were back-filled with 3 M KCl solution. As soon as a control measurement had been performed, electrodes were removed from the oocytes and cells were placed in small (10 mL) dishes for drug incubation (30 minutes). After incubation, cells were again placed in the recording chamber and the experiment was repeated. For the analysis of frequency dependence, cells were kept in the recording chamber after the control measurement without pulsing (at the holding potential). Midazolam was washed-in over a period of 15 minutes, and the measurements were repeated. For whole-cell patch-clamp experiments, the bath solution contained 140 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 10 mM HEPES, 1.8 mM CaCl₂, and 10 mM glucose (pH adjusted to 7.4 with NaOH). The pipette solution contained 100 mM K-aspartate, 10 mM KCl, 2 mM MgCl₂, 1 mM CaCl₂, 10 mM ethylene glycol tetraacetic acid (EGTA), 10 mM HEPES, and 40 mM glucose (pH adjusted to 7.2 with KOH). All electrophysiological measurements were carried out at room temperature (22°C).

Heterologous expression of Kv1.5 channel subunits in *Xenopus* oocytes

The Kv1.5 (GenBank accession number NM_002234) potassium channel clone was a generous gift of Dr B Wible (Cleveland, OH, USA). Out of the pSP64 plasmid, complementary Kv1.5-RNA was prepared using the mMACHINE[®] in vitro transcription kit (Ambion, Huntingdon, UK) by use of SP6 polymerase after linearization with EcoRI (Hoffman-La Roche Ltd.). RNA injection into stage V and VI defolliculated oocytes was performed using a Nanoject automatic injector (Drummond, Broomall, USA). *Xenopus* frogs were kept under species-appropriate conditions at the Interfaculty Biomedical Faculty (IBF) of the University of Heidelberg, Heidelberg, Germany. Frogs were anesthetized with a solution containing 0.3% ethyl-3-aminobenzoate-methanesulfonic acid (15°C) for oocyte extraction. As soon as 600 to 800 oocytes had been carefully removed, the muscle layer and skin were sutured and the animal was revitalized. The volume of injected complementary RNA (cRNA) solution was 46 nL per oocyte, and measurements were carried out 1 to 3 days after injection. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication No 85-23, revised 1996) and was approved by the regional administrative council (Regierungspräsidium Karlsruhe, Karlsruhe, Germany) (application number G-211/12).

Heterologous expression of Kv1.5 channels and immunostaining

Human embryonic kidney (HEK) 293 cells stably expressing Kv1.5 channels were obtained from CLS Cell Line Service (Eppelheim, Germany). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL penicillin G sodium, 100 µg/mL streptomycin sulfate, and 100 µg/mL gentamicin in an atmosphere of 95% humidified air and 5% CO₂ at 37°C. Cells were passaged regularly with trypsin/ethylenediaminetetraacetic acid (EDTA) and subcultured prior to treatment in 3 mL dishes.

Electrophysiological experiments and data analysis

Kv1.5 currents in *Xenopus* oocytes were obtained using the double-electrode voltage-clamp technique as reported previously.²¹ Microelectrodes had tip resistances ranging from 1 to 5 MΩ. Data were low-pass-filtered at 1 to 2 kHz (−3 dB, four-pole Bessel filter) before digitalization at 5 to 10 kHz. Recordings were performed using a commercially available amplifier (Warner OC-725A; Warner Instruments, Hamden, CT, USA) and the pCLAMP software package (Molecular Devices LLC, Sunnyvale, CA, USA) for data acquisition and analysis. For patch-clamp experiments, cells were transferred from the incubator into a recording chamber which was continuously rinsed with bath solution. For measurements, single cells were selected. Pipettes had resistances of 3–4 MΩ and whole-cell currents were measured with an RK-400 amplifier (Bio-Logic SAS, Claix, France) and analyzed with the pCLAMP software package. Dose-response curves were fitted to the Hill function:

$$Y = A1 + \{[A2 - A1]/[1 + 10^{\log(X_0 - X) \cdot n_H}]\}, \quad (1)$$

with A1 being the bottom asymptote, A2 the top asymptote, $\log X_0$ being the center, and n_H being the Hill slope. Activation and inactivation curves were fitted to a Boltzmann function:

$$Y = \{ \{ (A1 - A2) / [1 + (e^{(X - X_0)/k})] \} + A2 \}, \quad (2)$$

with A1 being the initial value, A2 being the final value, X_0 representing the half-maximal activation potential, Y the degree of activation, and k the slope factor. Statistical data are expressed as mean ± standard error, with “n” representing the number of experiments performed. Statistical significance

was evaluated using the independent Student's *t*-test or the analysis of variance (ANOVA), followed by the Bonferroni post hoc analysis where necessary. Differences were considered significant when the *P*-value was <0.05.

Results

Midazolam inhibits heterologously expressed Kv1.5 channels

Inhibitory effects of midazolam on cloned Kv1.5 channels were first analyzed using the double-electrode voltage-clamp technique in *Xenopus* oocytes. From a holding potential of −80 mV, cells were depolarized by a variable voltage step (1,250 ms) ranging from −90 to +50 mV (10 mV increments). When activated with the depolarizing voltage step, Kv1.5 channels gave rise to a rapidly activating and slowly inactivating outward potassium current, reminiscent of cardiac ultra-rapidly delayed rectifier current I_{Kur} . Figure 1A exemplarily displays a single Kv1.5 current trace elicited by a voltage step to +50 mV. Incubation with midazolam (100 µM) resulted in a reduction of Kv1.5 current (representative experiment of n=8). Under control conditions, Kv1.5 currents exhibited a small run-up to 131.6%±25.1% (n=9). Incubation with increasing midazolam concentrations (10, 30, 100, 200, 300, and 1,000 µM) resulted in a dose-dependent change of Kv1.5 current amplitude by +4.8%±12.4%, −12.3%±12.3%, −39.1%±14.3%, −72.0%±8.9%, −86.2%±10.7%, and −94.9%±3.4%, respectively (n=6–9). The IC₅₀ in the *Xenopus* oocyte expression system was 104.1±27.7 µM (n=6–9, n_H =1.66±0.66) (Figure 1B). To underline the relevance of the observed effects, inhibitory effects of midazolam were next analyzed in a mammalian cell line (HEK 293) stably expressing Kv1.5 channels using the patch-clamp technique. Kv1.5 current was elicited using a single-step voltage protocol (600 ms) ranging from +60 to −80 mV (20 mV decrements). Figure 1C displays a typical current trace elicited by a step to +60 mV before and after application of 30 µM midazolam. As expected, compared to the *Xenopus* expression system, applications of 1, 3, 10, 30, and 100 µM midazolam resulted in more pronounced reductions, of 16.2%±5.7%, 25.7%±6.4%, 34.9%±2.8%, 54.6%±2.7%, and 65.6%±11.6%, respectively. The IC₅₀ of inhibition was 17.0±4.2 µM (n=5–10, n_H =1.50±0.4) (Figure 1D).

Pharmacological properties of Kv1.5 channel inhibition

Effects of midazolam on biophysical properties of Kv1.5 channels were further analyzed using a double-step voltage

Figure 1 Midazolam inhibits cloned cardiac Kv1.5 potassium channels.

Notes: (A) Representative current trace of a typical Kv1.5 current elicited by a rectangular voltage protocol in *Xenopus* oocytes. Midazolam (100 μ M) results in a strong inhibition of Kv1.5 current. (B) Dose–response curves for inhibition of Kv1.5 by midazolam established in *Xenopus* oocytes. (C) Representative current trace of a typical Kv1.5 current elicited by a rectangular voltage protocol in an HEK cell line using the patch-clamp technique. Midazolam (30 μ M) results in a pronounced reduction of Kv1.5 current. (D) Dose–response curves established in an HEK cell line stably expressing Kv1.5 channels.

protocol. From a holding potential of -80 mV, cells were depolarized by a variable voltage step (1,250 ms) ranging from -90 to $+50$ mV (10 mV increments). This variable step was followed by a constant return pulse to $+50$ mV (50 ms) eliciting outward tail currents. Figure 2A and B display typical families of Kv1.5 current traces before and after application of midazolam (100 μ M) (see inset for voltage protocol). The corresponding current–voltage relationship measured at peak current amplitude (measured at the first dashed line in Figure 2A and B) is displayed in Figure 2C. Kv1.5 channel activation curves were established, as reported previously, by dividing current amplitude by the corresponding electrochemical driving force (Figure 2D).²¹ The I–V relationship of each cell before and after midazolam application was fitted with a Boltzmann function in order to obtain half-maximal activation voltages ($V_{1/2}$). Under

control conditions, Kv1.5 channels revealed a mean $V_{1/2}$ of 1.93 ± 0.85 mV ($n=6$) (Figure 2D). Inhibition by midazolam did not significantly influence the $V_{1/2}$ ($V_{1/2} = 3.21 \pm 1.47$ mV, $n=6$, $P>0.05$) (Figure 2D).

Channel inactivation curves were established by plotting tail current amplitude during the constant return pulse (measured at the second dashed line in Figure 2A and B) versus the test pulse potential of the preceding variable voltage step (Figure 2E). Half-maximal inactivation voltages before and after midazolam application were obtained by fitting the data with a Boltzmann function. Under control conditions, Kv1.5 channels revealed a mean half-maximal inactivation voltage of 7.15 ± 1.12 mV ($n=6$). Application of midazolam (100 μ M) resulted in a small but significant leftward shift of the mean half-maximal inactivation voltage ($V_{1/2} = 3.41 \pm 0.74$ mV, $n=6$, $P<0.05$) (Figure 2E).

Figure 2 Pharmacological properties of Kv1.5 current inhibition.

Notes: Typical families of Kv1.5 current traces elicited by a double-step voltage protocol (inset in [A]) before (A) and after (B) incubation with 100 μ M midazolam in *Xenopus* oocytes. (C) Current-voltage relationship of Kv1.5 current under control conditions (filled boxes) and after incubation with midazolam (open circles) measured at peak current ($n=6$). (D) Kv1.5 activation curves established by dividing peak current amplitude by the electrochemical driving force. Midazolam did not significantly influence the half-maximal activation voltage ($V_{1/2}$) ($n=6$). (E) Kv1.5 channel inactivation curves established by plotting tail current amplitude versus the potential of the first voltage step. Midazolam resulted in a small but significant shift of the inactivation curve ($n=6$).

Time course of block development

Block development was next analyzed using a single depolarizing voltage step to +50 mV. Kv1.5 current was measured under control conditions and after incubation with either 30 μ M or 1,000 μ M (Figure 3A and B). Incubation with 30 μ M and 1,000 μ M midazolam resulted in a current reduction of $12.25\% \pm 12\%$ and $94.91\% \pm 3\%$, respectively ($n=7$) (Figure 3A and B). Time course of block development could be obtained by dividing the current trace after midazolam incubation by the corresponding current trace under control conditions (Figure 3C and D). In both cases,

block development was fast, yielding a mean time constant of 9.0 ± 2.1 ms for 30 μ M midazolam (Figure 3C) and 4.1 ± 0.8 ms for 1,000 μ M midazolam (Figure 3D) (representative experiment of $n=6$).

Inhibition of Kv1.5 channels by midazolam exhibits no frequency dependence

Frequency dependence of block is a common finding among ion channel inhibitors. In order to analyze if Kv1.5 channels exhibit more pronounced inhibition at higher pacing rates,

Figure 3 Time constants of channel inhibition.

Notes: Exemplary current traces elicited by a rectangular voltage step to +50 mV under control conditions and after application of 30 μM (A) or 1,000 μM (B) midazolam in *Xenopus* oocytes. Time course of block development was determined by division. In both cases, development of block was fast, yielding time constants (τ) of 9.0 ms for 30 μM (C) and 4.1 ms for 1,000 μM (D) midazolam.

a set of three different voltage protocols was established. Cells were depolarized by a test pulse to +50 mV (200 ms) that was repeated with a frequency of 1, 2, or 4 Hz for duration of 16 seconds (Figure 4A, inset). Having obtained a control measurement, 200 μM midazolam was washed-in over a period of 15 minutes and the measurement was repeated. Peak current amplitude of each pulse before and after drug incubation was measured to determine the degree of block. Figure 4A–C displays the first (at 0 second) and the last (at 16 seconds) recordings after midazolam incubation for 1 Hz, 3 Hz, and 4 Hz (representative experiment of n=6 each). Figure 4D summarizes the inhibitory effects of midazolam on current amplitude. After 16 seconds, midazolam (200 μM) resulted in relative reductions of Kv1.5 current of $0.64\% \pm 0.033\%$, $0.66\% \pm 0.034\%$, and $0.67\% \pm 0.033\%$ for 1 Hz, 2 Hz, and 4 Hz, respectively (n=6). However, at this point in time, there was no significant frequency dependence of block ($P < 0.05$, ANOVA). In order to achieve a clear presentation, data are only presented for every full second (Figure 4D).

Discussion

We have shown that the anesthetic midazolam is a typical open-channel blocker of cardiac Kv1.5 potassium channels. So far, inhibitory effects of midazolam on Kv1.5 channels have not been described.

Pharmacological effects of midazolam

Pharmacological effects of midazolam on Kv1.5 channels were analyzed using two different heterologous expression systems (*Xenopus* and HEK 293). Compared to native cardiomyocytes, the main advantage of these simplified cell models mainly relates to the absence of interfering ion currents. Strategies aimed at blocking these currents, either by using elaborated voltage protocols or small molecule inhibitors, might negatively influence the current under analysis. Within several pharmacological studies, we were able to prove that the human kidney cell line HEK 293 represents a suitable cell model for the expression and analysis of cardiac ion channels.^{22–25} Midazolam inhibited cloned Kv1.5 channels in a dose-dependent manner, yielding an IC_{50} within the low micromolar range

Figure 4 Frequency dependence of Kv1.5 channel block.

Notes: Frequency dependence of block was analyzed in *Xenopus* oocytes using three different pacing rates (1 Hz, 2 Hz, and 4 Hz). Having obtained a control measurement, 300 μ M midazolam was washed-in and the measurement was repeated. (A–C) Exemplary experiments showing the first (t=0 seconds) and last (t=16 seconds) current traces after application of 300 μ M midazolam. (D) The degree of inhibition was calculated for the last test pulse (at 16 seconds), yielding no significant dependence on the pacing rate (n=6).

Abbreviations: t, time; s, seconds.

when analyzed in a mammalian cell line (HEK 293). As expected, when analyzed in *Xenopus* oocytes, we found that considerably higher midazolam concentrations were necessary to achieve similar effects. This phenomenon is well recognized and has been attributed to diffusion barriers within *Xenopus* oocytes caused by the vitelline membrane and yolk sac.²⁶ As a consequence, results obtained from the mammalian HEK cell line might be better suited to questioning the physiological relevance of our findings. The dose–response relationship obtained in these cells is in line with previously published data on pharmacological inhibition of ion channels by the anesthetic midazolam. For example, an IC_{50} of 10 μ M has been observed for calcium channels.²⁷ Similarly, the whole-cell potassium outward current I_K , measured in tracheal smooth muscle cells, as well as the calcium-independent transient outward current I_{to} , were reduced by approximately 20% after application of 100 or 60 μ M midazolam, respectively.^{12,27}

For the induction of anesthesia, the recommended intravenous midazolam dose is approximately 0.15 to 0.4 mg/kg.²⁸ Depending on individual metabolic variations, reported peak plasma concentrations during induction of anesthesia can vary

widely, and may reach values of up to 2 mg/L ($\sim 5 \mu$ M/L).^{29,30} Interestingly, some healthy subjects may exhibit prolonged elimination half-time of midazolam.³¹ This might be partly explained by individual differences in the activity of hepatic enzymes, namely the members of the cytochrome P450 enzyme family, 3A4 and 3A5.³² Furthermore, in patients with renal or hepatic dysfunction, clearance from blood may be reduced, resulting in high blood levels over long time periods.³³ When analyzed in a mammalian cell line, we found that application of 3 μ M midazolam resulted in a reduction of Kv1.5 current of 25.7%. Considering the reported peak plasma levels of $\sim 5 \mu$ M/L, these effects seem small. However, it has to be taken into account that midazolam acts as a multichannel inhibitor. Only recently, So et al reported inhibitory effects of midazolam on a neural delayed rectifier potassium current $I_{K(DR)}$.¹³ We recently observed that midazolam acts as a low-affinity inhibitor of cardiac iou channel gene (hERG) channels (Vouderlin et al, unpublished data, 2014). Considering these additional effects, it is tempting to speculate that inhibitory effects on cardiac potassium channels might act in an additive manner. On the other hand, midazolam has also been shown

to interfere with depolarizing ion channels.^{13,27} Simultaneous inhibition of depolarizing and repolarizing ion channels might cancel each other out, thereby accounting for the obvious gap between positive in vitro data and the paucity of in vivo evidence. Action potential recordings obtained from human atrial myocytes would help with analysis of the net effect of midazolam on atrial electrophysiology. Taking all evidence, due to the complex electrophysiological mechanisms, definite conclusions of midazolam-induced effects on the shape and duration of cardiac action potential can only be drawn with great caution. From the in vitro and in vivo data available, the use of midazolam seems safe in clinical practice and the clinical relevance of its side effects on cardiac electrophysiology seem negligible under therapeutic dosages. However, the data presented herein add to the current understanding of the pharmacological profile of midazolam. Furthermore, only a few small molecule inhibitors of Kv1.5 have been reported so far. Within this context, midazolam might help in the identification of new lead compounds for the development of structurally new Kv1.5 inhibitors.

Decrease of Kv1.5 current could be due either to a decrease in open probability or a decrease of the number of available channels. However, when analyzing properties of Kv1.5 channel inhibition in detail, we found that midazolam exhibits a fast onset of block. From the kinetics of block development, it can be concluded that inhibition of the Kv1.5 channel is state dependent, and does not occur in the closed state. A similar behavior was recently reported for the inhibition of Kv1.5 channels by the antiarrhythmic ajmaline.²¹ These effects strongly favor an inhibition via a decrease in open probability due to channel blockade. We further showed that midazolam caused a shift of the Kv1.5 channel inactivation curve. However, this shift was small, and the physiological relevance of this observation remains to be confirmed.

Midazolam did not alter channel activation kinetics and induced no frequency dependence of block. Analyzing binding sites of structurally unrelated Kv1.5 channel inhibitors, Decher et al were able to identify a number of critical amino acid residues located within the pore helix as well as in the S6 domain.^{34,35} Interestingly, they further showed that the exact binding site is dependent on the size and shape of the inhibitor with larger molecules (eg, AVE0118) interfering with biophysical properties of the channel ("trapping mechanism").³⁴ Following this hypothesis, it is tempting to speculate that midazolam, similarly to the Kv1.5 inhibitor S0100176, might bind to amino acids located within the central cavity of Kv1.5 channels.³⁴ Accordingly, no pronounced effects on channel gating were observed. However, this hypothesis needs to be

further validated by alanine-scanning mutagenesis. Taking all evidence midazolam represents a typical open channel inhibitor of cardiac Kv1.5 currents that does not have marked effects on channel gating kinetics.

Study limitations

The transferability of the present results might be limited by technical shortcomings. For example, all experiments were conducted at room temperature. However, it is well recognized that ion channel function is temperature dependent. Therefore, it cannot be excluded that pharmacological effects might differ slightly when analyzed at physiological temperature. Furthermore, it is well recognized that midazolam acts as a multichannel inhibitor with reported inhibitory effects on both depolarizing and repolarizing ion currents. Thus, the net effect of midazolam on cardiac electrophysiology is expected to be complex and cannot simply be derived from observed effects on isolated ion channels. Action potential recordings obtained in isolated human cardiomyocytes would aid in further understanding of the sum effect.

Conclusion

We have shown that the anesthetic midazolam is a typical open-channel blocker of cardiac Kv1.5 channels. Our findings add to the current understanding of the pharmacological profile of the compound midazolam. These data may assist with identification of new lead compounds for the development of Kv1.5 inhibitors.

Acknowledgments

This work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG) Scho1350/2-1 Zi1177/1-1 to EPS and EZ. FF was supported by the Hartmut Hoffmann-Berling International Graduate School of Molecular and Cellular Biology. The skillful assistance of Christine Jeckel is gratefully acknowledged. DS was supported by a grant from the University of Heidelberg: Postdoc Fellowship Program of the Faculty of Medicine.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Thummel KE, O'Shea D, Paine MF, et al. Oral first-pass elimination of midazolam involves both gastrointestinal and hepatic CYP3A-mediated metabolism. *Clin Pharmacol Ther.* 1996;59(5):491–502.
2. Nordt SP, Clark RF. Midazolam: a review of therapeutic uses and toxicity. *J Emerg Med.* 1997;15(3):357–365.
3. Nonaka A, Kashimoto S, Imamura M, Furuya A, Kumazawa T. Mechanism of the negative inotropic effect of midazolam and diazepam in cultured foetal mouse cardiac myocytes. *Eur J Anaesthesiol.* 1997;14(5):481–487.

4. Study RE, Barker JL. Diazepam and (–)-pentobarbital: fluctuation analysis reveals different mechanisms for potentiation of gamma-aminobutyric acid responses in cultured central neurons. *Proc Natl Acad Sci U S A*. 1981;78(11):7180–7184.
5. Morani G, Bergamini C, Angheben C, et al. General anaesthesia for external electrical cardioversion of atrial fibrillation: experience of an exclusively cardiological procedural management. *Europace*. 2010;12(11):1558–1563.
6. Wutzler A, Loehr L, Huemer M, et al. Deep sedation during catheter ablation for atrial fibrillation in elderly patients. *J Interv Card Electrophysiol*. 2013;38(2):115–121.
7. Wutzler A, Mueller A, Loehr L, et al. Minimal and deep sedation during ablation of ventricular tachycardia. *Int J Cardiol*. 2014;172(1):161–164.
8. Hüneke R, Fassl J, Rossaint R, Lückhoff A. Effects of volatile anesthetics on cardiac ion channels. *Acta Anaesthesiol Scand*. 2004;48(5):547–561.
9. Staikou C, Stamelos M, Stavroulakis E. Impact of anaesthetic drugs and adjuvants on ECG markers of torsadogenicity. *Br J Anaesth*. 2014;112(2):217–230.
10. Miró O, de la Red G, Fontanals J. Cessation of paroxysmal atrial fibrillation during acute intravenous propofol administration. *Anesthesiology*. 2000;92(3):910.
11. Michaloudis DG, Kanakoudis FS, Petrou AM, Konstantinidou AS, Pollard BJ. The effects of midazolam or propofol followed by suxamethonium on the QT interval in humans. *Eur J Anaesthesiol*. 1996;13(4):364–368.
12. Buljubasic N, Marijic J, Berczi V, Supan DF, Kampine JP, Bosnjak ZJ. Differential effects of etomidate, propofol, and midazolam on calcium and potassium channel currents in canine myocardial cells. *Anesthesiology*. 1996;85(5):1092–1099.
13. So EC, Wu KC, Kao FC, Wu SN. Effects of midazolam on ion currents and membrane potential in differentiated motor neuron-like NSC-34 and NG108-15 cells. *Eur J Pharmacol*. 2014;724:152–160.
14. Nerbonne JM. Molecular basis of functional voltage-gated K⁺ channel diversity in the mammalian myocardium. *J Physiol*. 2000;525 Pt 2: 285–298.
15. Kanto JH. Midazolam: the first water-soluble benzodiazepine. Pharmacology, pharmacokinetics and efficacy in insomnia and anesthesia. *Pharmacotherapy*. 1985;5(3):138–155.
16. Feng J, Wible B, Li GR, Wang Z, Nattel S. Antisense oligodeoxynucleotides directed against Kv1.5 mRNA specifically inhibit ultrarapid delayed rectifier K⁺ current in cultured adult human atrial myocytes. *Circ Res*. 1997;80(4):572–579.
17. Wettwer E, Hála O, Christ T, et al. Role of IK_{ur} in controlling action potential shape and contractility in the human atrium: influence of chronic atrial fibrillation. *Circulation*. 2004;110(16):2299–2306.
18. Li GR, Feng J, Yue L, Carrier M, Nattel S. Evidence for two components of delayed rectifier K⁺ current in human ventricular myocytes. *Circ Res*. 1996;78(4):689–696.
19. Tibbs GR, Rowley TJ, Sanford RL, et al. HCN1 channels as targets for anesthetic and nonanesthetic propofol analogs in the amelioration of mechanical and thermal hyperalgesia in a mouse model of neuropathic pain. *J Pharmacol Exp Ther*. 2013;345(3):363–373.
20. López-Izquierdo A, Ponce-Balbuena D, Ferrer T, Rodríguez-Menchaca AA, Sánchez-Chapula JA. Thiopental inhibits function of different inward rectifying potassium channel isoforms by a similar mechanism. *Eur J Pharmacol*. 2010;638(1–3):33–41.
21. Fischer F, Vonderlin N, Zitron E, et al. Inhibition of cardiac Kv1.5 and Kv4.3 potassium channels by the class Ia anti-arrhythmic ajmaline: mode of action. *Naunyn Schmiedeberg's Arch Pharmacol*. 2013;386(11):991–999.
22. Fischer F, Vonderlin N, Seyler C, et al. Acute and subacute effects of the selective serotonin-noradrenaline reuptake inhibitor duloxetine on cardiac hERG channels. *Naunyn Schmiedeberg's Arch Pharmacol*. 2013;386(9):795–804.
23. Scholz EP, Konrad FM, Weiss DL, et al. Anticholinergic antiparkinson drug orphenadrine inhibits HERG channels: block attenuation by mutations of the pore residues Y652 or F656. *Naunyn Schmiedeberg's Arch Pharmacol*. 2007;376(4):275–284.
24. Scholz EP, Alter M, Zitron E, et al. In vitro modulation of HERG channels by organochlorine solvent trichloromethane as potential explanation for proarrhythmic effects of chloroform. *Toxicol Lett*. 2006;165(2):156–166.
25. Zitron E, Scholz E, Owen RW, et al. QTc prolongation by grapefruit juice and its potential pharmacological basis: HERG channel blockade by flavonoids. *Circulation*. 2005;111(7):835–838.
26. Madeja M, Musshoff U, Speckmann EJ. Follicular tissues reduce drug effects on ion channels in oocytes of *Xenopus laevis*. *Eur J Neurosci*. 1997;9(3):599–604.
27. Yamakage M, Matsuzaki T, Tsujiguchi N, Honma Y, Namiki A. Inhibitory effects of diazepam and midazolam on Ca²⁺ and K⁺ channels in canine tracheal smooth muscle cells. *Anesthesiology*. 1999;90(1):197–207.
28. Reves JG, Fragen RJ, Vinik HR, Greenblatt DJ. Midazolam: pharmacology and uses. *Anesthesiology*. 1985;62(3):310–324.
29. Crevat-Pisano P, Dagna S, Granthil C, Coassolo P, Cano JP, Francois G. Plasma concentrations and pharmacokinetics of midazolam during anaesthesia. *J Pharm Pharmacol*. 1986;38(8):578–582.
30. Persson P, Nilsson A, Hartvig P, Tamsen A. Pharmacokinetics of midazolam in total i.v. anaesthesia. *Br J Anaesth*. 1987;59(5):548–556.
31. Dundee JW, Halliday NJ, Harper KW, Brogden RN. Midazolam. A review of its pharmacological properties and therapeutic use. *Drugs*. 1984;28(6):519–543.
32. Wandel C, Böcker R, Böhler H, Browne A, Rügheimer E, Martin E. Midazolam is metabolized by at least three different cytochrome P450 enzymes. *Br J Anaesth*. 1994;73(5):658–661.
33. Kulka PJ, Lauven PM, Schüttler J, Apffelstaedt C. Methohexital vs midazolam/flumazenil anaesthesia during laryngoscopy under jet ventilation. *Acta Anaesthesiol Scand Suppl*. 1990;92:90–95; discussion 107.
34. Decher N, Kumar P, Gonzalez T, Pirard B, Sanguinetti MC. Binding site of a novel Kv1.5 blocker: a “foot in the door” against atrial fibrillation. *Mol Pharmacol*. 2006;70(4):1204–1211.
35. Decher N, Pirard B, Bundis F, et al. Molecular basis for Kv1.5 channel block: conservation of drug binding sites among voltage-gated K⁺ channels. *J Biol Chem*. 2004;279(1):394–400.

Drug Design, Development and Therapy

Publish your work in this journal

Drug Design, Development and Therapy is an international, peer-reviewed open-access journal that spans the spectrum of drug design and development through to clinical applications. Clinical outcomes, patient safety, and programs for the development and effective, safe, and sustained use of medicines are a feature of the journal, which

Submit your manuscript here: <http://www.dovepress.com/drug-design-development-and-therapy-journal>

has also been accepted for indexing on PubMed Central. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Dovepress