Ketamine and propofol differentially inhibit human neuronal \( K^+ \) channels

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Summary

Interaction of intravenous anaesthetic agents with voltage-dependent potassium channels significantly correlates with clinical concentrations. If potassium channels were to play an important part in anaesthesia, one might expect different effects at the molecular level of those anaesthetics that show different clinical effects. Whole cell patch-clamp experiments were analysed in detail in order to compare the effects of two clinically diverse intravenous hypnotics, ketamine and propofol, on voltage-dependent potassium channels in human neuroblastoma SH-SYSY cells. Both anaesthetics inhibited the potassium conductance in a concentration-dependent and reversible manner with \( IC_{50} \) values of 300 \( \mu \)M and 45 \( \mu \)M for ketamine and propofol respectively. Whereas ketamine shifted the midpoint of current activation by maximally 14 mV to more hyperpolarized potentials, propofol had the opposite effect on the activation midpoint. Current inhibition by ketamine increased with voltage but decreased with propofol at higher membrane potentials. Propofol but not ketamine induced concentration-dependent but voltage-independent decline, akin to inactivation, of the voltage-dependent potassium channels. Thus, the anaesthetics differ not only in their clinical profiles but they also show differential actions on voltage-dependent potassium channels in several ways. This provides additional evidence for the hypothesis that voltage-dependent potassium channels play an important role in anaesthesia.

Keywords: anaesthetics, intravenous, ketamine, propofol; anaesthetic mechanisms, \( K^+ \) channels.

Introduction

Recent unexpected evidence demonstrates that inhibition of voltage-dependent \( K^+ \) currents by intravenous \( i.v \) anaesthetics significantly correlates with clinical concentrations [1]. Furthermore, voltage-dependent \( K^+ \) channels are suppressed at clinically relevant concentrations [1]. Investigating the inhibition of these ion channels may not only help to establish molecular determinants of anaesthetic potency [1] but half-maximal inhibition of these \( K^+ \) channels also seems to predict the clinical potency of anaesthetic agents [1]. The interaction of general anaesthetics with this class of ion channels, thus, warrants further analysis. In view of the excellent correlation between clinical anaesthesia and in vitro action on human \( K^+ \) channels [1] it would be important to establish if inhibition of \( K^+ \) channels may also allow discrimination between clinically divergent anaesthetic agents. If, as suggested by the correlation with clinical potency, \( K^+ \) channels were to play an important part in anaesthesia one would expect different effects at the molecular level of those anaesthetics that show different clinical effects.

Ketamine produces a unique anaesthetic state, described as 'dissociative anaesthesia' [2]. It is manifest clinically as a spontaneously breathing cataleptic patient with open eyes, intact light corneal reflexes, spontaneous movements unrelated to surgical stimuli and a satisfactory state of hypnosis and analgesia [3]. Propofol is widely used both for induction and maintenance of general anaesthesia [4] without exerting 'dissociative' or analgesic actions. The effect of ketamine is attributed to its influence on N-methyl-D-aspartate (NMDA)-receptors [5] and that of

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This document contains a scientific study discussing the effects of propofol on potassium and calcium currents in neurons. The study involves the use of electrophysiological recordings to measure voltage-sensitive currents in neurons. The data analysis and conclusions are presented to support the findings. The text includes methods for culturing cells, preparing the cells for recordings, and analyzing the results. The study was conducted by P. Friederich et al. in 2001.
as $1 - g_{\text{Kmax}}$ in the presence of the drug in relation to the mean of $g_{\text{Kmax}}$ under control conditions and after wash out of the drug effect. Inhibition of steady-state current after 50–60 ms by propofol is also given. This was necessary as inhibition of K conductance and steady-state current differed akin to induction induced by propofol but not by ketamine. Concentration-dependent shifts of $V_{\text{m}}$ were measured as the difference between $V_{\text{m}}$ in the presence of the drug and the mean of $V_{\text{m}}$ under control conditions and after wash out of the drug effect. The concentration-response curves were established using non-linear regression [11] and Sigma-Plot 4.0 Software (Sigm-Plot, Jandel Scientific, Erkrath, Germany). The measured inhibition of $g_{\text{Kmax}}$ and the measured shift of $V_{\text{m}}$, where fitted to the Hill equation: $\ln(V/V_{\text{m}}) = c'/(IC_{50} + c')$. Here $b$ = block of $g_{\text{Kmax}}$ or shift of $V_{\text{m}}$, $B_{\text{max}}$ = maximal block of $g_{\text{Kmax}}$ or maximal shift of $V_{\text{m}}$, $c = concentration$, $\gamma = Hill$ coefficient and $IC_{50} = concentration$ of half-maximal effect. Time-constants of anaesthetic-induced K current decline were established by fitting monoeponential functions to the K currents after they had reached their maximum. Statistical comparison was performed with paired or unpaired Student's t-test as appropriate and considered significant for $P \leq 0.01$. Data are always shown as mean ± SEM.

Results

Original recordings of the K currents under control condition, under propofol and ketamine, and after the wash out of the drugs are shown in Fig. 1. Propofol and ketamine differentially affected the time course of the currents during the test pulse (Fig. 1). Propofol caused a pronounced current decline or inactivation-like behaviour that was not observed under control condition. This decline was observed at all membrane potentials between +10 mV and +70 mV. The time constants of the anaesthetic-induced decline were dependent on drug concentration but not on voltage. Time-constants ranged between 2 and 5 ms at membrane potentials of maximal current activation (+40 to +70 mV) and they were more than 100 times faster compared with time constants of K current inactivation without propofol ($n = 31$).

The induction of inactivation-like behaviour by propofol allowed discrimination between inhibition of peak current at the beginning of the test pulse and inhibition of the K current after 50–60 ms of the test pulse. In order to quantify this time-dependent effect the ratio of maximal current inhibition to current inhibition after 50–60 ms of the test pulse ($I_{\text{ISO-60}}$) was calculated for both anaesthetics at concentrations that caused nearly 50% inhibition of the K currents. Whereas ketamine (250 µM) suppressed current maxima and $I_{\text{ISO-60}}$ equally as well (47 ± 2% vs. 45 ± 1%, mean ± SEM, $n = 5$, $P \geq 0.01$), inhibition of $I_{\text{ISO-60}}$ by propofol (43 µM) was 1.6 times higher ($P \leq 0.01$) than inhibition of the current maxima (44.9 ± 0.02% vs. 29.9 ± 0.02%, mean ± SEM, $n = 9$, $P \leq 0.01$).

For the comparison of the action of both drugs, current-voltage curves were generated for inhibition of the maximal outward currents and subsequently converted to conductance-voltage curves (see Methods). This allowed comparison of the action of both drugs independent of time. Additionally, a separate analysis of concentration-dependent effects of ketamine and propofol on K conductance and current activation was possible. The maximal K conductance ($G_{\text{Kmax}}$) and the midpoints of current activation ($V_{\text{m}}$) of the control currents did not differ significantly between ketamine and propofol experiments. $G_{\text{Kmax}}$ for the control currents of ketamine and propofol experiments was 3.75 ± 0.16 and 4.31 ± 0.17 (mean±SEM, $n = 26$, 34, $P > 0.01$). $V_{\text{m}}$ was 7.54 ± 1.31 and 7.00 ± 0.69 for the respective experiments (mean±SEM, $n = 25$, 34, $P > 0.01$). Both anaesthetics inhibited $G_{\text{Kmax}}$ in a concentration-dependent and reversible manner (Fig. 2, white symbols). As estimated by their respective $IC_{50}$-value, ketamine inhibited $G_{\text{Kmax}}$ 5–6 times more potently than propofol. The concentration-response curve for inhibition of $I_{\text{ISO-60}}$ by propofol is also shown in order to illustrate the time-dependent drug effect of propofol (Fig. 2, black symbols, data taken from [1]).

Propofol, as well as ketamine, shifted the midpoint of current activation along the voltage axis in a concentration-dependent and reversible manner (Fig. 3). $V_{\text{m}}$ was, however, shifted in opposing directions by both drugs. Whereas propofol caused a depolarizing shift of $V_{\text{m}}$ by maximally 15 mV, ketamine maximally shifted $V_{\text{m}}$ by the same amount towards more hyperpolarized membrane potentials (Fig. 3). The voltage dependence of conductance block as analysed at concentrations of propofol and ketamine close to their respective $IC_{50}$-values differed between both anaesthetics, as well. Inhibition of the K conductance by propofol decreased with increasing test potentials. This decrease could be described by regression analysis. The slope of the regression line was $-1.3$ and the regression coefficient $r^2$ was 0.9 ($n = 7$). In contrast to propofol, inhibition by ketamine increased with voltage. This increase could best be described with a function of...
exponential rise (inhibition = a(1-e^{-b})) with values for a and b of 4.9 s^{-1} and 1.4 e^{-1}, respectively; \textit{r}^2 = 0.9, n = 5).

**Discussion**

It had previously been shown that i.v. anaesthetics, including propofol and ketamine, reversibly inhibited human neuronal K currents in SH-SYSY cells. This suppression correlated with their clinical concentrations [1]. The detailed analysis presented in this paper provides evidence that propofol and ketamine differ in their actions on potassium currents in several ways. The pattern of diverse pharmacological action contradicts the view of non-specific anaesthetic interaction of propofol and ketamine with this family of human K channels. Both drugs differ in their effects on current activation (Fig. 3) that would lead to opposite effects on the threshold of action potentials. As a result of the inactivation-like behaviour, propofol will have a greater impact on the refractory time than ketamine when inhibition is normalized to peak current inhibition (Figs 1 and 2). Furthermore, as the membrane potential changes during an action potential the different voltage-dependence of inhibition would lead to different effects of propofol and ketamine on the shape of the action potential.

Systemic consequences of molecular effects are difficult to predict from in vitro experiments. However, the results show that diverse clinical profiles are reflected already at the molecular level of human K channels. SH-SYSY cells express voltage-dependent K channels of the Kv3.1 subtype [12]. Kv3.1 channels are, for example, expressed in GABAergic interneurons of the hippocampus [13]. As these ion channels are critical for the integra-

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At first, the small effects at clinically relevant concentrations may suggest that inhibition of this type of voltage-dependent K channel is not involved in pharmacological action during clinical anaesthesia. However, it is increasingly recognized that small effects on the molecular level may be modulated on the way up the integrative structure of the entire central nervous system [15,16]. Ion channel proteins are integrated with other membrane proteins within a single cell. Single cells form neuronal networks with other cells, and these neuronal networks in turn are integrated into higher functional units. A concentration-response curve at the bottom of this hierarchical structure does not need to be identical to the concentration-response curve at the top [15,16].

Anaesthetic agents exert their effects at each level of the central nervous system (Fig. 4). At the molecular level, the anaesthetic effects are described by the right most concentration-response curve. Because the neuronal output of the lower level is the input of the next higher level (Fig. 4), the concentration-response curve of the higher level would be multiplied with the concentration-response curve of the preceding level [15,16]. Therefore, depending on the level of integration, the composite concentration-response curve will constitute the result of an increasing
In summary, inhibition of human neuronal K channels by propofol and ketamine resulted from qualitative distinct effects. Ketamine and propofol differ not only in their clinical profiles but they also show differential actions at the molecular level. Our results provide further support for the hypothesis that Kv3.1 channels constitute a molecular target relevant for clinical anaesthesia.

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