

## Stereospecific Interaction of Ketamine with Nicotinic Acetylcholine Receptors in Human Sympathetic Ganglion-like SH-SY5Y Cells

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**Background:** Surprising clinical evidence suggests a block of sympathetic transmission by ketamine. The action of ketamine on nicotinic acetylcholine receptors (nAChRs) in human ganglions is unknown. Because ganglionic transmission depends on nAChRs, such information may help to clarify whether ketamine impairs ganglionic transmission in men. Because racemic ketamine as well as *S*(+)-ketamine are used clinically, the authors investigated stereospecific effects on human ganglionic nAChRs. Stereospecific psychomimetic effects have been attributed to voltage-dependent Kv channel inhibition; therefore the effects on nAChRs were compared with those on Kv channels present in the same cells.

**Methods:** Whole-cell currents through nAChRs and K channels were measured in SH-SY5Y cells with the patch-clamp technique by application of acetylcholine (1 mM, nAChRs) or by a step depolarization from a holding potential of  $-80$  mV to  $+40$  mV (K channels). Electrolyte conditions were identical for both currents.

**Results:** Racemic ketamine and the isomers inhibited nAChRs and K channels in a concentration-dependent and reversible manner. Racemic ketamine inhibited nAChRs and K channels, with the anesthetic concentration inducing the half-maximal effect being  $1.4$  and  $300$   $\mu$ M, respectively. Only inhibition of the nAChRs was stereoselective. The half-maximal concentrations

were  $0.8$  and  $3.6$   $\mu$ M for *S*(+)- and *R*(-)-ketamine. The K channels were  $350$  and  $70$  times less sensitive to the effects of *S*(+) and *R*(-)-ketamine.

**Conclusion:** Ketamine at concentrations found during clinical anesthesia exerts stereospecific effects on human ganglionic nAChRs but not on voltage-dependent K channels. Our results support the view that ketamine impairs sympathetic ganglionic transmission. Nonspecific effects on voltage-dependent K channels may underlie psychomimetic side effects. (Key words: Ganglionic transmission; human K channels; intravenous anesthesia; patch-clamp technique.)

CONTRARY to expectations, muscle sympathetic nerve discharge decreases during ketamine anesthesia in volunteers.<sup>1</sup> A centrally mediated sympathomimetic action either is not present in man or is not transmitted to the periphery. This view is supported by measurements of finger plethysmograms in surgical patients that indirectly indicated a ganglionic blocking effect of the dissociative anesthetic.<sup>2</sup> Direct evidence about the action of ketamine on ganglionic transmission in the human sympathetic nervous system is lacking and difficult to obtain. A concentration-dependent depression of sympathetic ganglionic transmission has been described in guinea pigs in an isolated hypogastric nerve–vas deferens preparation.<sup>3</sup>

Ganglionic transmission critically depends on the stimulation of postsynaptic ganglionic nicotinic acetylcholine receptors (nAChRs). The ganglionic depression in animal preparations<sup>3</sup> has been attributed to antinicotinic ergic action. The effect of ketamine on human ganglionic nAChRs is unknown; such knowledge would provide important data to assess whether ketamine may have ganglionic blocking effects in man. Investigating the isomers of ketamine may provide information on differential effects of the isomers on ganglionic transmission.

The human peripheral neuroblastoma cell line SH-SY5Y<sup>4</sup> resembles sympathetic neurons in culture.<sup>5,6</sup> This cell line constitutes a model of a sympathetic ganglion in which the effect of ketamine on human ganglionic nAChRs can be investigated. Apart from nAChRs these

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cells also express Kv3 potassium channels.<sup>7</sup> Inhibition of Kv channels has been implicated repeatedly in psychomimetic side effects of ketamine.<sup>8,9</sup> At equivalent anesthetic concentrations *S*(+)-ketamine exhibits less psychomimetic side effects than racemic ketamine.<sup>10</sup> Therefore, if inhibition of Kv channels contributes to psychomimetic side effects of ketamine, the action on Kv channels should not be as stereoselective. SH-SY5Y cells offer the unique opportunity to compare the actions of ketamine on two molecular targets that both may be modified during ketamine anesthesia.

This study was designed primarily to investigate the effect of ketamine on nAChRs in a model of human sympathetic ganglion cells. The results of our study may elucidate further whether ketamine, contrary to expectations, induces impairment of sympathetic ganglionic transmission in man. The effect of ketamine isomers on Kv channels also was investigated.

## Methods

### Cell Culture

SH-SY5Y cells were grown in nonconfluent monolayer using RPMI medium (Biochrom, Berlin, Germany) at 37°C with 95% air and 5% CO<sub>2</sub>. Growth medium contained 10% fetal calf serum (Biochrom), penicillin (100 U/ml), and streptomycin (100 µg/ml) (Life Technologies, Paisley, Scotland). Neuronal differentiation was induced by exposure to 10 µM retinoic acid (Sigma, Deisenhofen, Germany) for 3–7 days.<sup>11</sup>

### Electrophysiology

Whole-cell patch-clamp recordings<sup>12</sup> were performed with an EPC7 amplifier (List, Darmstadt, Germany). nAChR currents were evoked by applying 1 mM acetylcholine for 1 s at a holding potential of –60 mV. The concentration of acetylcholine (Sigma) was chosen because it results in a maximal current response of the nAChRs. K currents were evoked by depolarizing the cell membrane from a holding potential –80 mV to +40 mV for 600 ms. These potentials were chosen because at –80 mV K channels do not show steady-state inactivation, and at a test potential of +40 mV they are fully activated.<sup>13</sup> Extracellular solution consisted of the following: NaCl: 150 mM; KCl: 5.6 mM; CaCl<sub>2</sub>: 1.8 mM; MgCl<sub>2</sub>: 1 mM; HEPES: 10 mM; pH: 7.4. Recording pipettes with resistances of 2.5–4.0 MΩ were filled with intracellular solution: KCl: 140 mM; EGTA: 10 mM; MgCl<sub>2</sub>: 5 mM; HEPES: 10 mM; pH: 7.4 mM. All constituents of the intra- and extracellular fluids were purchased from Sigma. The

experimental procedure was chosen to allow a comparison of both current systems under identical conditions. Both channels were fully activated by the experimental protocol; they reside in the same membrane environment; and they were measured under identical electrolyte conditions. This is important as both the lipid and the electrolyte environment may influence anesthetic action.<sup>14,15</sup> Patch pipettes were prepared from borosilicate glass capillaries (Kwik-fil; WPI, Saratoga, FL) with a two-stage pipette puller and a microforge (L/M-3P-A and L/M CPZ-101, List). Experiments were performed at room temperature (21–23°C).

### Application of Agonist and Drugs

The effects of anesthetic agents on the nAChR currents were studied in a continuous exposure of the measured cell to the drug before and during application of acetylcholine (1 mM acetylcholine for all ketamine experiments) or the voltage step (equilibrium condition). With 1 mM acetylcholine one can record the currents with a good signal-to-noise ratio. Although it might be interesting to know the acetylcholine dependence of the ketamine effect, lower acetylcholine concentrations would induce much smaller currents and thus result in much poorer signal-to-noise ratios. Perfusion was achieved with a fast-application system (NPI, Tamm, Germany) solution exchange in < 10 ms). Ketamine and the isomers were a kind gift from Goedecke (Freiburg, Germany). The purity of the isomers was above 99%. Drug solutions were prepared daily from aqueous stocks. Recorded data were stored on a personal computer.

### Data Analysis and Statistics

Current analysis was performed with pClamp software (version 6.0.4, Clampfit, Axon Instruments, Foster City, CA). Inhibition was measured as peak current inhibition of nAChRs and steady-state current inhibition of K channels. The peak nAChR current was chosen, because it is more physiologically relevant. Because ketamine did not show time-dependent effects on the K channels and the K channels do not exhibit fast inactivation the time during the test pulse for measuring K channel inhibition by ketamine was uncritical. Concentration-response curves were fitted to Hill equations (Graph Pad Inplot 4.03, Graph Pad, San Diego, CA); confidence intervals of the fit parameters are quoted as specified by the Graph Pad software. The Hill equation is

$$i = c^n / (c^n + IC_{50}^n),$$

in which *i* is the blocked current as fraction of the

control current,  $c$  is the concentration of the anesthetic,  $n$  is the Hill coefficient (equal to 1, unless stated otherwise), and  $IC_{50}$  is the anesthetic concentration inducing the half-maximal effect. Comparisons between concentration-response curves were made using the F test.<sup>16</sup> Experiments were included if a washout of  $\geq 90\%$  was obtained. Values are the mean  $\pm$  SD unless stated otherwise.  $P \leq 0.05$  was considered statistically significant.

### Clinical Concentrations

Free plasma concentrations during ketamine anesthesia are reported to be around  $15 \mu\text{M}$  for racemic ketamine, with mean plasma concentrations of  $S(+)$ - and  $R(-)$ -ketamine twofold below and twofold above, respectively, those of racemic ketamine<sup>10</sup> (for a discussion of free plasma concentrations during clinical anesthesia see also ref. 13). Values as high as  $60$ – $100 \mu\text{M}$  occasionally have been considered clinically relevant.<sup>17</sup>

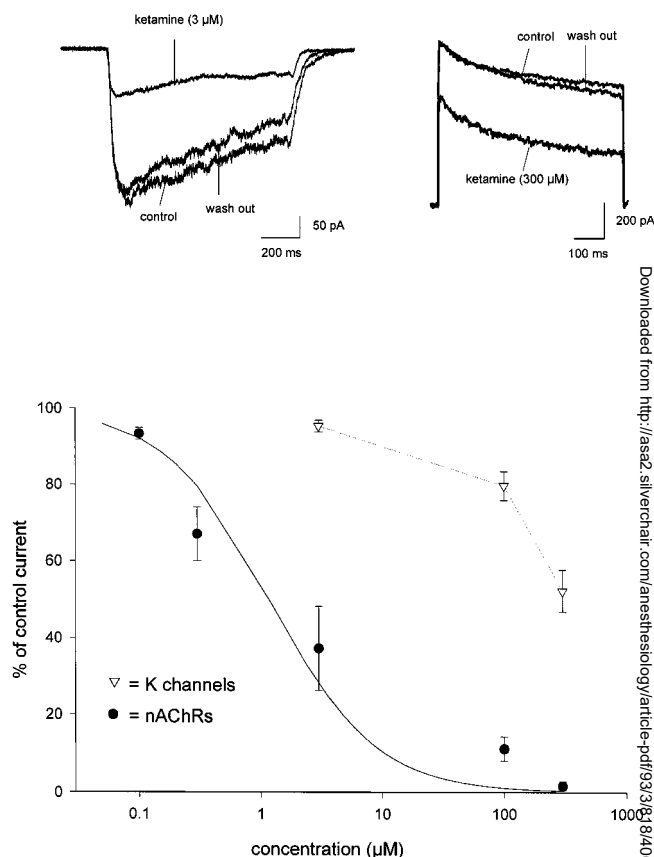
## Results

The nAChR-mediated currents show an agonist concentration-response curve characterized by a Hill coefficient of 1.9 and an  $EC_{50}$  value for acetylcholine of  $100 \mu\text{M}$ . Acetylcholine at  $1 \text{ mM}$  caused maximal current activation and induced peak whole-cell currents of  $144 \pm 16 \text{ pA}$  ( $n = 25$  cells). Ketamine did not induce currents in SH-SY5Y cells. Depolarizing the cell membrane from  $-80 \text{ mV}$  to  $+40 \text{ mV}$  resulted in a K current amplitude of  $803 \pm 288 \text{ pA}$  after  $500$ – $600 \text{ ms}$  of the test pulse ( $n = 48$  cells).

### Effects of Racemic Ketamine

Racemic ketamine suppressed acetylcholine-induced currents in a concentration-dependent and reversible manner. Figure 1 shows original current recordings of nAChR currents before and during the application of ketamine, as well as after washout. The size of the mean acetylcholine-induced peak current under control conditions was  $273 \pm 95 \text{ pA}$  ( $n = 18$  cells). The concentration-dependent inhibitory effects of ketamine on peak nAChR current were fitted by a Hill function (fig. 1). The half-maximal concentration was  $1.37 \mu\text{M}$  (95% confidence interval:  $0.76$ – $2.16$ ;  $n = 17$  cells).

Although the primary intent was to investigate possible stereospecific effects of ketamine on Kv channels, we also performed measurements of K current inhibition by racemic ketamine. We used identical electrolyte conditions as for the nAChR experiments to exclude any

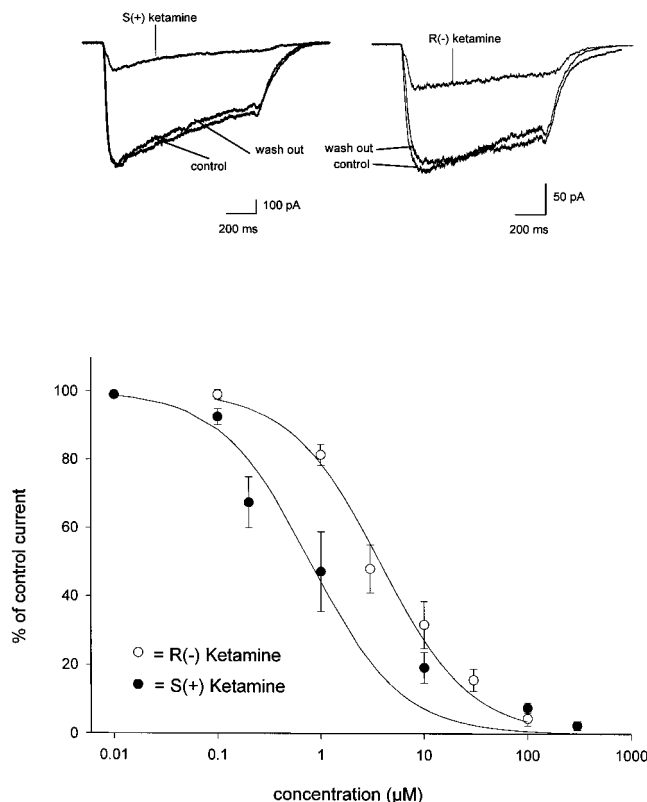


**Fig. 1. Racemic ketamine reversibly inhibits nicotinic acetylcholine receptors (nAChRs; top left) and K channels (top right) present in the same membrane with different potencies. Current recordings show currents induced by acetylcholine ( $1 \text{ mM}$ ) under control conditions, during the application of ketamine ( $3 \mu\text{M}$ ), and after washout of the drug. K currents were recorded by depolarizing the membrane from a holding potential of  $-80 \text{ mV}$  to  $+40 \text{ mV}$ . Shown are the K currents under control condition, during the ketamine effect ( $300 \mu\text{M}$ ), and after washout of the drug. Concentration-dependent effects on nAChRs were described mathematically by a Hill function. The concentration for half-maximal inhibition was  $1.4 \mu\text{M}$ ; the Hill coefficient was  $-0.7$ . A complete concentration-response curve for K current inhibition was not obtained because data already existed.<sup>13</sup>  $IC_{50} = 361 \pm 39 \mu\text{M}$ ; Hill coefficient =  $0.9 \pm 0.1$  (mean  $\pm$  SEM).**

influence of the ion composition on inhibitory effects. Current recordings before and during the application of ketamine and after washout of the drug are shown in figure 1. These experiments confirmed our previous results, revealing a half-maximal effect of racemic ketamine on the K channels at around  $300 \mu\text{M}$ .

### Effects of $S(+)$ - and $R(-)$ -ketamine

The  $S(+)$ -isomer of ketamine suppressed acetylcholine-induced currents in a concentration-dependent and



**Fig. 2.** *S*(+)-ketamine (top left) and *R*(-)-ketamine (top right) reversibly inhibit nicotinic acetylcholine receptors (nAChRs). Recordings show currents induced by acetylcholine (1 mM) under control conditions, during the application of *S*(+)-ketamine (10 μM) and *R*(-)-ketamine (10 μM), and after the wash-out of the drugs. The  $IC_{50}$  value for current inhibition by the *S*(+)-isomer was three- to fourfold lower than that of the *R*(-)-isomer. Concentration-dependent effects on nAChRs were described mathematically by Hill functions. The parameters of the Hill function for the *S*(+)-isomer were as follows:  $IC_{50}$  value = 0.92 μM, Hill coefficient = -0.7; for the *R*(-)-isomer:  $IC_{50}$  value = 3.6 μM, Hill coefficient = -0.9. The  $IC_{50}$  values for nAChR inhibition significantly differed between the *S*(+)- and the *R*(-)-isomer.

reversible manner. Figure 2 shows original current recordings of nAChR currents before, during, and after the application of *S*(+)-ketamine. The size of the acetylcholine-induced currents under control conditions was  $378 \pm 392$  pA (range 40–1596,  $n = 29$  cells). Fitting the concentration-dependent inhibitory effects of *S*(+)-ketamine on peak nAChR current with a Hill function (fig. 2) yielded a concentration for half-maximal effect of 0.78 μM (95% confidence interval: 0.60–1.02 μM,  $n = 29$  cells). The *R*(-)-isomer of ketamine again suppressed acetylcholine-induced currents in a concentration-dependent and reversible manner (fig. 2). The average size of the acetylcholine-induced current in these experi-

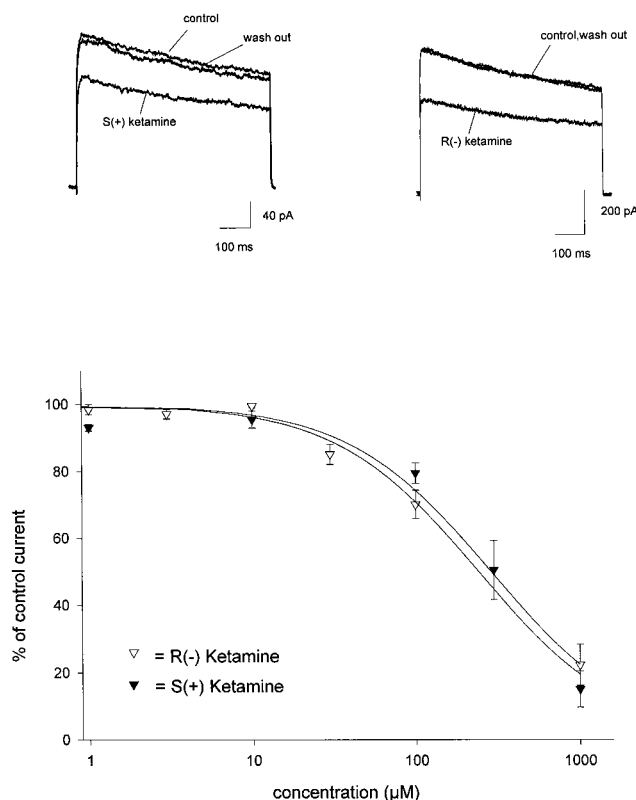
ments under control conditions was  $361 \pm 121$  pA (range 166–533,  $n = 17$  cells). The function resulting from a Hill fit to the concentration-dependent effects of *R*(-)-ketamine on peak nAChR current is depicted in figure 2. The  $IC_{50}$  value was 3.58 μM (95% confidence interval: 2.95–4.35 μM,  $n = 17$ ). As judged by their  $IC_{50}$  values, the *S*(+)-isomer of ketamine was a significantly more potent inhibitor of the nAChRs than the *R*(-)-isomer.

We further investigated whether the effects of the *S*(+)- and *R*(-)-ketamine on voltage-operated K channels present in SH-SY5Y membranes also may be stereospecific. K currents were measured under the same electrolyte conditions as the acetylcholine-induced currents. The amplitude of the K currents were  $829 \pm 41$  pA (range 330–1,790 pA,  $n = 19$  cells) and  $922 \pm 21$  pA (range 589–1,400 pA,  $n = 17$  cells) for the experiments with *S*(+)- and *R*(-)-ketamine, respectively. Both isomers inhibited the K currents in a concentration-dependent and reversible manner (fig. 3;  $n = 25$  and 22 cells for *S*(+)- and *R*(-)-ketamine, respectively). Neither isomer induced inactivation-like behavior of the K currents in SH-SY5Y cells. In contrast to inhibition of nAChRs, both isomers inhibited the K channels with identical potency (fig. 3). The  $IC_{50}$  values were 70 and 350 times greater than  $IC_{50}$  values of *R*(-)- and *S*(+)-ketamine for nAChR inhibition, respectively.

## Discussion

Inhibitory effects of racemic ketamine and ketamine isomers were compared between nAChRs and voltage-dependent K channels present in human neuroblastoma SH-SY5Y cells. The much higher sensitivity of nAChRs in comparison to the K channels already suggested a more specific interaction of ketamine with the ligand-gated nAChRs, compared with the voltage-operated K channels. A specific protein-mediated action of ketamine on human nAChRs is substantiated by the stereospecificity of the inhibitory effect. The *S*(+)-isomer is four to five times more potent an inhibitor than the *R*(-)-isomer and two times more potent than the racemic mixture. In contrast to the nAChRs, voltage-dependent K channels in SH-SY5Y are inhibited by ketamine without any stereoselectivity. K channel suppression, therefore, results from a nonspecific effect on the K channel protein that may be mediated by the lipid membrane or hydrophobic parts of the protein. The differences in anesthetic potency and stereoselectivity between inhibition





**Fig. 3.** *S*(+)-ketamine (top left) and *R*(-)-ketamine (top right) reversibly inhibit voltage-dependent K channels. K currents were recorded by depolarizing the membrane from a holding potential of  $-80$  mV to  $+40$  mV. Shown are the K currents under control conditions, during the application of *S*(+)- and *R*(-)-ketamine ( $100$   $\mu$ M), and after washout of the drugs. Concentration-dependent effects on K channels were described mathematically by Hill functions. The  $IC_{50}$  values and Hill coefficients for K channel inhibition were, respectively,  $286$   $\mu$ M and  $-1.3$  for the *S*(+)-isomer, and  $250$   $\mu$ M and  $-0.9$  for the *R*(-)-isomer. The  $IC_{50}$  values for K channel inhibition did not differ between the isomers.

of nAChRs and Kv channels does not result from systematic variations of the experimental set-up. Both channels reside in the same membrane and were measured under identical electrolyte conditions. Stereospecific effects on ganglionic nAChRs and nonstereospecific pharmacologic action on voltage-dependent K channels may contribute to side effects observed during ketamine anesthesia.

The human neuroblastoma cell line SH-SY5Y resembles sympathetic neurons in culture.<sup>5</sup> As analyzed by polymerase chain reaction SH-SY5Y cells contain cDNA of  $\alpha 3$ ,  $\alpha 4$ ,  $\alpha 5$ ,  $\alpha 7$ ,  $\beta 2$ ,  $\beta 3$ , and  $\beta 4$  nAChR subunits. Northern blot experiments confirm the presence of mRNA of  $\alpha 3$ ,  $\alpha 7$ ,  $\beta 2$ , and  $\beta 4$  nAChR subunits with intensity of the hybridization signal of  $\alpha 3 > \beta 4 > \beta 2 > \alpha 7$ .<sup>18</sup> This expression pattern corresponds well with

data recorded by Wang *et al.*,<sup>19</sup> who demonstrated that most of the functional nAChRs in the SH-SY5Y cell line consist of an  $\alpha 3$ - $\beta 2$  and  $\alpha 3$ - $\beta 4$  subunit combination. The same nAChR subunits can be detected in sympathetic ganglion cells of rat and chicken and in the rat PC12 cell line.<sup>20,21</sup> Human SH-SY5Y cells, like for example chick ciliary ganglion neurons,<sup>6</sup> contain mRNA of  $\alpha 7$  subunits,<sup>18,22</sup> but their level of expression is low.<sup>18,23</sup> The nAChR subunits in SH-SY5Y cells, like in chick ciliary ganglion, assemble to acetylcholine receptors that do not bind  $\alpha$ -bungarotoxin.<sup>22,23</sup> Despite more abundant expression of  $\alpha 3$ - $\beta 2$  and  $\alpha 3$ - $\beta 4$  nAChR subunits in the peripheral nervous system, these subunits are not limited to the periphery,<sup>24</sup> and pharmacologic effects may be attributed to interaction with these subunits present in central neurons, as well. The  $IC_{50}$  value for nAChR inhibition in SH-SY5Y cells by racemic ketamine of  $1.4$   $\mu$ M reflects a 10-fold higher affinity of these nAChRs than of those in rat PC12 cells.<sup>25</sup> This difference in ketamine sensitivity may be explained by the use of different agonists in the studies, nicotine *versus* acetylcholine, or perhaps more importantly by species differences. Human neuronal nAChR  $\beta 3$  and  $\beta 4$  subunits, for example, only show 94 and 89% amino acid sequence identity with the rat neuronal nAChR  $\beta 3$  and  $\beta 4$  subunits, respectively.<sup>18,24,26</sup>

Suppression of nAChR currents in human ganglion cells would impair ganglionic transmission and may lead to cardiovascular depression. Ketamine impairs cardiovascular function in critically ill patients.<sup>27,28</sup> This side effect has been explained by direct action on myocardial ion channels.<sup>29,30</sup> These *in vitro* effects of ketamine on myocardial ion channels occur at concentrations higher than clinical,<sup>30</sup> and there remains considerable uncertainty as to whether they underlie ketamine-induced cardiovascular depression.<sup>30</sup> Our results demonstrate inhibitory effects on ganglionic nAChRs at concentrations much lower than necessary for inhibition of myocardial ion channels. Clinical evidence for a ganglionic blocking effect of ketamine were established by finger plethysmograms.<sup>2</sup> These experiments demonstrated ketamine-induced peripheral vasodilatation that is incompatible with sympathetic stimulation and that has been explained by a ganglionic block.<sup>2</sup> Suppression of nAChRs may offer an additional explanation for why ketamine depresses cardiovascular function in some patients.

The anesthetic effects of ketamine are stereospecific<sup>10</sup> and have been suggested to result from interaction with *N*-methyl-D-aspartate receptors.<sup>31</sup> The potency ratio of *S*(+)- and *R*(-)-ketamine at the *N*-methyl-D-aspartate re-

ceptor and the ganglionic nAChRs are very similar. As a consequence, both isomers as well as the racemic mixture would lead to similar effects on ganglionic nAChRs at clinically equivalent anesthetic concentrations. Our results suggest that, with regard to ganglionic transmission, neither isomer would have an advantage over the racemic mixture in critically ill patients.

Inhibition of voltage-dependent K channels has been implicated repeatedly in the psychomimetic action of ketamine.<sup>8,9</sup> Because the lack of stereospecific action on voltage-dependent K channels, the ratio of specific NMDA receptor suppression to nonspecific K channel suppression would be different for the *S*(+)- and the *R*(-)-isomers. Because *S*(+)-ketamine is a significantly more potent anesthetic it should have less inhibitory action on the K channel than *R*(-)-ketamine if used at clinically equivalent anesthetic concentrations. It is tempting to speculate that different psychomimetic side effect profiles of both isomers<sup>10</sup> may be caused in part by different degrees of nonspecific interactions with voltage-operated K channels.<sup>8,9</sup> It remains a matter of debate, however, whether this degree of ion channel alteration is sufficient to result in clinically observable effects.<sup>32,33</sup>

Ketamine stereospecifically inhibits neuronal nAChRs in a human model of sympathetic ganglion cells. The effects occur with EC<sub>50</sub> values well within clinically relevant concentrations and suggest that specific interaction with these ion channel proteins may cause clinical side effects sometimes observed during ketamine anesthesia. In contrast to psychomimetic side effects that may be caused by inhibition of voltage-dependent Kv channels, neither isomer nor racemic ketamine would be advantageous with regard to ganglionic blockade.

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