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Synergistic Inhibition of Muscarinic Signaling by Ketamine Stereoisomers and the Preservative Benzethonium Chloride

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Background: **Ketamine (Ketalar; Parke-Davis, Morris Plains, NJ) has been shown to inhibit muscarinic signaling with a median inhibitory concentration (IC_{50}) of 5.7 μM. Whereas Ketalar is a racemic mixture, recent interest has focused on clinical use of the S(+) ketamine isomer, which is three times as potent an analgesic as the R(−) isomer yet seems to be associated with fewer psychoactive side effects. Therefore, the authors studied the effects of S(+) and R(−) ketamine and the preservative benzethonium chloride on muscarinic signaling.**

Methods: **Rat m1 muscarinic acetylcholine receptors were expressed recombinantly in *Xenopus laevis* oocytes. Ca^{2+}-activated Cl^- currents in response to 10^{-7} M acetyl-β-methylcholine were determined by two-electrode voltage clamping in the presence of various concentrations of ketamine and benzethonium. Concentration-inhibition curves were constructed and used for algebraic and isobolographic analysis.**

Results: **The IC_{50} was 125 ± 33 μM for S(+) ketamine, and 91 ± 19 μM for R(−) ketamine. This difference was not statistically significant, indicating that muscarinic inhibition by ketamine is not stereoselective. The R(−)/S(+) mixture had an IC_{50} of 48 ± 1 μM, and thus the stereoisomers interact synergistically. When appropriate concentrations of benzethonium were added, an IC_{50} of 15 ± 2 μM resulted.**

Conclusions: **The muscarinic inhibitory action of ketamine isomers is not stereoselective. Because S(+) ketamine is a significantly more potent analgesic, it should have less muscarinic inhibitory action than R(−) ketamine when used in clinically equivalent doses. A significant fraction of the muscarinic inhibitory action of Ketalar is due to the preservative benzethonium. If reconstituted with a different preservative, Ketalar might be a less potent muscarinic antagonist. (Key words: Receptors, muscarinic acetylcholine. Anesthetics, intravenous: ketamine; benzethonium chloride. *Xenopus* oocytes. Stereoselectivity. Cell signaling.)**

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PHENCYCLIDINES have been shown to interact with muscarinic acetylcholine receptors,[1–5] and previously we showed that the phencyclidine derivative ketamine also has muscarinic antagonist activity.[6] Although ketamine's primary site of action is the N-methyl-D-aspartate receptor,[7,8] secondary actions of the compound might be relevant to the unique anesthetic state it induces. In particular, the inhibitory actions of ketamine on muscarinic signaling may have clinical relevance: In addition to explaining some of the compound's side effects (such as the excess sympathetic tone and bronchodilation observed after ketamine administration), they may be relevant to its anesthetic properties. Recent studies have shown that central muscarinic systems play profound roles in learning, maintenance of consciousness, and pain perception,[9] and that inhibition of these pathways results in states resembling sleep.

Our previous study[6] used Ketalar, the commercial form of ketamine available from Parke-Davis (Morris Plains, NJ). It is a racemic mixture of the two stereoisomers of ketamine, S(+) and R(−), and it also contains the preservative benzethonium chloride (BCl). Recently, interest has developed in using S(+) ketamine for clinical purposes because it is approximately three times as potent an analgesic as R(−) yet is associated with fewer of the troublesome psychoactive side effects.[10,11] Thus we thought that a study of the muscarinic inhibitory actions of the two ketamine isomers would be appropriate. We considered two possible outcomes of the investigation. If muscarinic inhibitory potencies of the ketamine isomers were found to correlate with their anesthetic potencies, this would suggest that muscarinic antagonism plays a role in the anesthetic action of the compound. If so, anesthetic action and muscarinic side effects probably could not be dissociated. In contrast, if muscarinic inhibitory potency and anesthetic potency did not correlate, this would suggest the possibility that the ketamine structure could be modified to obtain anesthetics with fewer muscarinic side effects.

Thus we studied the effects of S(+) and R(−) ketamine and that of the preservative BCl on the functioning of m1 muscarinic acetylcholine receptors recombinantly expressed in *Xenopus* oocytes. The specific goals of the study were 1) to determine if inhibition of muscarinic acetylcholine signaling by ketamine isomers is stereoselective, and 2) if so, to determine if muscarinic inhibitory potencies correlate with those observed for anesthetic action.

Materials and Methods

Oocyte Harvesting

The study protocol was approved by the Animal Research Committee at the University of Virginia. Female *Xenopus laevis* toads were obtained from Xenopus I (Ann Arbor, MI), housed in an established colony, and fed regular frog brittle twice weekly. For removal of oocytes, an animal was immersed in an 8-mM solution of tricaine until unresponsive to a painful stimulus (toe pinching). A 1-cm-long incision was made in a lower abdominal quadrant, and a lobule of ovarian tissue, containing approximately 200 oocytes, was removed and placed in modified Barth's solution (containing 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 0.3 mM $Ca_2(NO_3)_2$, 15 mM HEPES, and 0.1 mg/ml gentamicin, with the *p*H adjusted to 7.6). The wound was closed in two layers, and the animal was allowed to recover in a separate tank overnight.

The ovarian tissue was washed immediately with modified Barth's solution and dissected into small clusters of 20–50 oocytes. Mature oocytes (Dumont stage V and VI) were isolated, and the follicular layer was removed manually from each oocyte using microforceps.

Receptor Expression

The rat m1 muscarinic acetylcholine receptor was a gift of T. I. Bonner (National Institutes of Mental Health, Bethesda, MD). Its complementary DNA (cDNA) consists of a 2.8-kilobasepair fragment in a commercial vector (pGEM1; Promega, Madison, WI). The construct was linearized by digestion with the nuclease *Hind*III, and complementary RNA (cRNA) was transcribed *in vitro* using the bacteriophage RNA polymerase T7. A capping analog (^{7m}GpppG) was included in the reaction to generate capped transcripts because these are translated more efficiently in the oocyte. The resulting cRNA was quantified by spectroscopy and diluted in water to a concentration of 0.1 mg/ml. Each oocyte was injected with 5 ng cRNA using an automated microinjector (Nanoject; Drummond Scientific, Broomall, PA). Adequacy of injection was confirmed by noting the slight increase in cell size during injection. The cells were cultured in modified Barth's solution at 18°C for 3 days before study.

Electrophysiologic Study

A single defolliculated oocyte was placed in a perfusable recording chamber (3-ml volume) filled with Tyrode's solution (containing 150 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM dextrose, 10 mM HEPES, with the *p*H adjusted to 7.4), with appropriate concentrations of ketamine isomers or BCl. Microelectrodes were pulled in one stage from capillary glass (BBL with fiber; World Precision Instruments, Sarasota, FL) on a micropipette puller (model 700C; David Kopf Instruments, Tujunga, CA). Tips were broken to a diameter of approximately 10 μm, providing a resistance of 1–3 MΩ. Electrodes were filled with 3 M KCl.

The cell was voltage clamped using a two-microelectrode oocyte voltage clamp amplifier (OC725C; Warner Corp., New Haven, CT) connected to a data acquisition and analysis system running on an IBM-compatible personal computer. The acquisition system consisted of a DAS-8 A/D conversion board (Keithley-Metrabyte, Taunton, MA), and analysis was performed with OoClamp software.[12] Holding potential was −70 mV. Cells that did not show a stable holding current of less than 1 μA during a 1-min equilibration period (fewer than 5% of cells tested) were excluded from analysis. Membrane current was sampled at 125 Hz and recorded for 5 s before and 55 s after application of the test compound. Acetyl-β-methylcholine (10^{-7} M) was applied to the oocyte in a 30-μl aliquot for 1 or 2 s using a micropipettor positioned approximately 5 mm from the oocyte. Each oocyte received only a single agonist application.

Data Analysis

Intracellular Ca^{2+} release in the *Xenopus* oocyte leads to opening of endogenous Ca^{2+}-activated Cl^- channels and a resulting inward Cl^- current ($I_{Cl(Ca)}$),[6,13] which can be used as a measure of agonist-induced Ca^{2+} release. Responses were quantified by integrating the current trace by quadrature and are reported as charge movements in units of microCoulombs (μC) because this reflects Ca^{2+} release better than peak current does.[14]

Responses are reported as means ± SEM. Concentration-inhibition curves were fitted to the Hill equation, and Hill coefficients and median inhibitory concentra-

tion (IC_{50}) values and their 95% confidence intervals were calculated. These were used for algebraic (fractional) and isobolographic analysis of drug interaction, using standard techniques.[15-17] Briefly, the most commonly used form of isobolographic analysis assumes that the effect of a combination of drugs without synergistic interaction is the sum of the effects of its constituents [$E_{(a+b)} = E_{(a)} + E_{(b)}$].[17,18] Therefore, we plotted the deviation of the IC_{50} of a mixture of two compounds from the line of additivity (*i.e.*, a straight line joining the two single-drug IC_{50}s) together with the 95% confidence interval. To determine if the difference between the IC_{50} of the mixture and the line of additivity was significant, we performed a *t* test. Algebraic analysis was performed as follows. At IC_{50} for the mixture, we determined, for each compound present, the ratio (r_c) of its concentration in the mixture to its IC_{50} when used alone. The r_c values for all compounds present in the mixture were then added (Σr_c).[17,18] If the interaction is additive, Σr_c should be 1. If Σr_c is smaller than 1, then potentiation (or superadditivity) of inhibition is present. If Σr_c is larger than 1, then antagonism (or subadditivity) of inhibition is present. R, the inverse of Σr_c, commonly is used to describe the strength of synergistic interactions. Probability values less than 0.05 were considered significant.

Materials

Molecular biology reagents were obtained from Promega (Madison, WI). All other chemicals were obtained from Sigma (St. Louis, MO). Ketamine isomers were supplied by Parke-Davis GmbH (Berlin, Germany).

Results

Expression of Muscarinic Acetylcholine Receptors in Oocytes

Whereas control oocytes were unresponsive to 10^{-7} M acetyl-β-methylcholine (MCh; data not shown), oocytes injected with cRNA encoding the m1 muscarinic acetylcholine receptor responded to MCh with a transient inward current (fig. 1A). This current developed within several seconds after agonist application, and membrane permeability returned to baseline after approximately 30 s. Average response sizes were 5.4 ± 0.5 μC. These findings are similar to those in our previous reports,[6,13] in which we showed these induced currents to be $I_{Cl(Ca)}$.

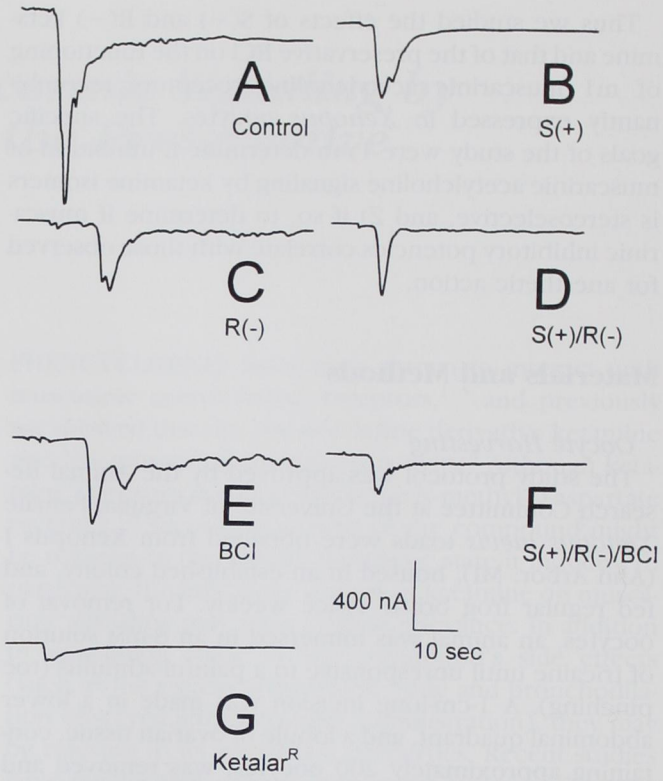

Fig. 1. Representative voltage clamp traces of *Xenopus* oocytes exposed to various combinations of ketamine and benzethonium chloride (BCl). Each trace is from a different cell. All cells were voltage clamped at −70 mV, and the agonist was 10^{-7} M acetyl-β-methylcholine in each instance. Ketamine concentrations were chosen to reflect approximately the median inhibitory concentration of the racemic mixture, and BCl concentrations were those present in 50 μM Ketalar. Bath solutions were (*A*) control (Tyrode's solution), 4.8 μC; (*B*) S(+) ketamine (50 μM), 3.1 μC; (*C*) R(−) ketamine (50 μM), 2.4 μC; (*D*) equimolar S(+)/R(−) ketamine (50 μM), 1.3 μC; (*E*) BCl (0.3 μM), 3.3 μC; (*F*) equimolar S(+)/R(−) ketamine (50 μM) plus BCl 0.3 μM), 0.8 μC; and (*G*) Ketalar (50 μM), 0.5 μC.

Ketamine Stereoisomers Synergistically Inhibit Muscarinic Signaling

We tested the ability of S(+) and R(−) ketamine to inhibit $I_{Cl(Ca)}$ induced by 10^{-7} M MCh in oocytes expressing m1 receptors. Oocyte responses were depressed in the presence of either compound. Representative traces are shown in figures 1B and C, and figures 2A and B show concentration-inhibition curves, obtained by fitting the experimental data to the Hill equation. Calculated IC_{50}s were 125 ± 33 μM for S(+) and 91 ± 19 μM for R(−) ketamine (table 1). Hill coefficients were 1.1 for each drug. The IC_{50} values were not significantly different, indicating that the muscarinic inhibitory action of ketamine on muscarinic receptor signaling is not stereoselective.

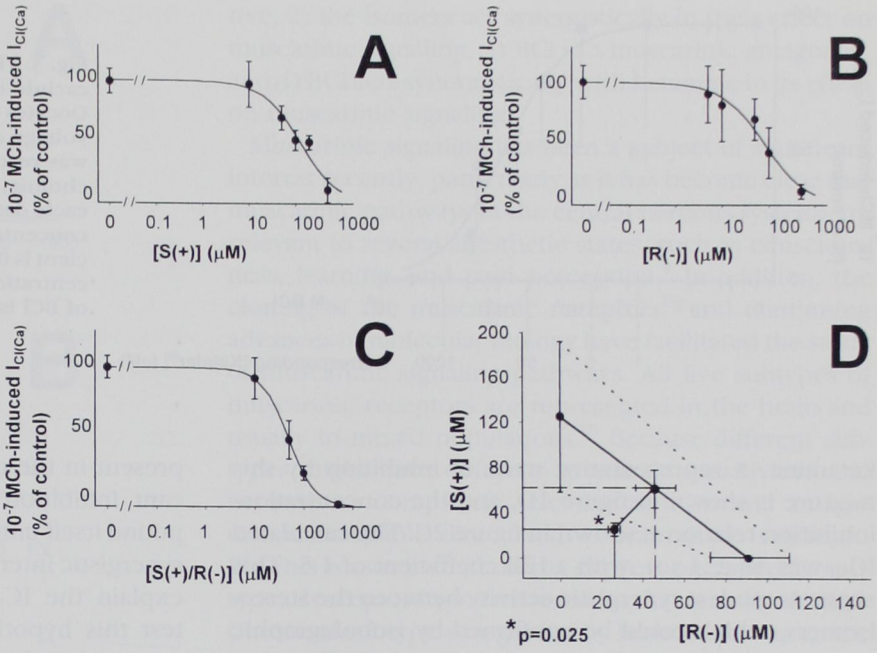

Fig. 2. The muscarinic inhibitory activity of S(+) and R(−) ketamine. Oocytes expressing the m1 muscarinic receptor were voltage clamped at −70 mV, and inward Cl^- current was recorded in response to 10^{-7} M acetyl-β-methylcholine. At least five oocytes were used to determine each data point. (*A*) The concentration-inhibition relation of S(+) ketamine. The calculated median inhibitory concentration (IC_{50}) is 125 ± 33 μM, and the Hill coefficient is 1.1. (*B*) The concentration-inhibition relation of R(−) ketamine. The calculated IC_{50} is 91 ± 19 μM, and the Hill coefficient is 1.1. (*C*) The concentration-inhibition relation of equimolar S(+)/R(−) ketamine. The calculated IC_{50} is 48 ± 1 μM, and the Hill coefficient is 1.5. (*D*) An isobologram of the interaction between S(+) and R(−) ketamine. The interaction is synergistic, and R is 2.2. *P = 0.025.

In our previous study,[6] we observed inhibition of muscarinic signaling by Ketalar with a calculated IC_{50} of 6 μM. Because the IC_{50} values from the individual stereoisomers were so different from that obtained with the commercial preparation, we hypothesized that a synergistic inhibitory action might exist between S(+) and R(−) ketamine. Therefore, we determined the inhibitory potency of an equimolar mixture of S(+) and (R−)

Table 1. Algebraic Analysis of the Interactions Between S(+) Ketamine, R(−) Ketamine, and BCl, Compared with Ketalar

Mixture	Component	Concentration of Component Present at IC_{50} of Mixture [μM (95% CI)]		r_c	Σr_c	R
S(+)	S(+)	125.0	(57.8, 192.2)	1.00	1.00	1.00
R(−)	R(−)	91.5	(53.3, 129.7)	1.00	1.00	1.00
BCl	BCl	0.88	(0.39, 1.37)	1.00	1.00	1.00
S(+)/R(−)	S(+)	24.0	(23.2, 24.8)	0.19		
					0.45	2.2*
	R(−)	24.0	(23.2, 24.8)	0.26		
S(+)/R(−)/BCl	S(+)/R(−)	14.9	(10.8, 19.0)	0.31		
					0.40	2.5†
	BCl	0.08	(0.06, 0.10)	0.09		
Ketalar	(S+)/R(−)	7.2	(0.0, 15.0)	0.15		
					0.20	5.0‡
	BCl	0.04§	(0.03, 0.05)	0.05		

r_c = ratio between the component's concentration in the mixture to its IC_{50} when used alone; Σr_c = sum of r_c values for all components in the mixture.

* $P = 0.025$.

† $P = 0.016$.

‡ $P < 0.001$.

§ Assuming a BCl concentration of 0.1 mg/ml in Ketalar stock solution.

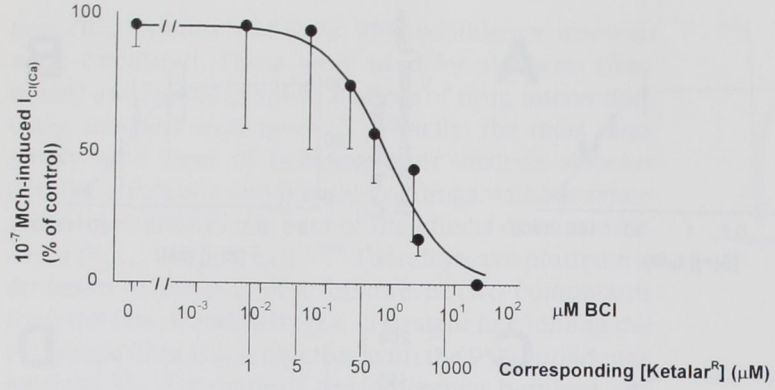

Fig. 3. The concentration-inhibition relation of muscarinic inhibition by benzethonium chloride (BCl). Oocytes expressing the m1 muscarinic receptor were voltage clamped at −70 mV, and inward Cl^- current was recorded in response to 10^{-7} M acetyl-β-methylcholine. At least five oocytes were used to determine each data point. The calculated median inhibitory concentration is 0.88 ± 0.25 μM, and the Hill coefficient is 0.9. The indicated corresponding Ketalar concentration is that in which the studied concentration of BCl is present.

ketamine. A representative trace of inhibition by this mixture is shown in figure 1D, and the concentration-inhibition relation is shown in figure 2C. The calculated IC_{50} was 48 ± 1 μM, with a Hill coefficient of 1.5. This suggests modest synergistic activity between the stereoisomers, which could be confirmed by isobolographic analysis (fig. 2D). Algebraic analysis revealed a Σr_c value of 0.45 and an R value of 2.2, which was significantly different ($P = 0.025$) from additivity (table 1).

Nonetheless, the calculated IC_{50} of the reproduced racemic mixture was still nearly a factor of 10 removed from that observed with Ketalar. Therefore, we studied the influence of the preservative in the mixture.

Benzethonium Chloride Is a Muscarinic Antagonist

Benzethonium chloride is present at concentrations of not more than 0.1 mg/ml in the stock solution of Ketalar, which contains 10 mg/ml racemic ketamine. Because BCl shows structural similarity to synthetic muscarinic antagonists, we hypothesized that the compound might have an inhibitory action on muscarinic signaling. Therefore, we determined the ability of BCl to inhibit $I_{Cl(Ca)}$ in response to 10^{-7} M MCh in oocytes expressing m1 muscarinic receptors. A representative trace is shown in figure 1E, and the concentration-inhibition relation is presented in figure 3. The calculated IC_{50} for the compound is 0.88 ± 0.25 μM (table 1), making it a more potent muscarinic antagonist than ketamine. The Hill coefficient for the concentration-inhibition relation was 0.9.

Benzethonium Chloride Acts Synergistically with Ketamine

However, at a concentration of racemic ketamine of approximately 50 μM (IC_{50}), only 0.3 μM BCl will be present in the commercial preparation, making significant inhibition of muscarinic signaling by the compound itself unlikely. Therefore, we hypothesized that a synergistic interaction between ketamine and BCl might explain the IC_{50} of 6 μM observed with Ketalar. To test this hypothesis, we reproduced the commercial preparation by mixing equimolar concentrations of S(+) and R(−) ketamine with the appropriate concentration of BCl and tested the resulting mixture for muscarinic inhibitory ability. A representative trace of its inhibitory activity is shown in figure 1F, and the concentration-response relation is shown in figure 4A. The reproduced mixture inhibited muscarinic signaling with a calculated IC_{50} of 15 ± 2 μM and a Hill coefficient of 0.6. This IC_{50} value is close to that previously observed with the commercial mixture.

As this suggests a synergistic interaction between ketamine and BCl, we subjected our data to isobolographic analysis, as shown in figure 4B. This analysis confirmed that ketamine and BCl act synergistically. As indicated in table 1, Σr_c was 0.40, and R was 2.5, similarly indicating synergistic interaction ($P = 0.016$).

Finally, to ascertain that our previous findings with Ketalar were reproducible, we determined its muscarinic inhibitory activity. A representative trace is shown in figure 1G, and the concentration-inhibition relation is shown in figure 4C. Ketalar inhibited MCh-induced $I_{Cl(Ca)}$ with an IC_{50} of 7.2 ± 3.8 μM, which is remarkably similar to our previously reported findings and not significantly different from the data obtained with our reproduced mixture. The Hill coefficient was 0.7. If the concentration of BCl in Ketalar stock solution was assumed to be 0.1 mg/ml, Σr_c was 0.20, and R was 5.0, which is significantly different from additivity ($P < 0.001$; table 1). This is a conservative estimate of the synergism; if the concentration of BCl was actually less

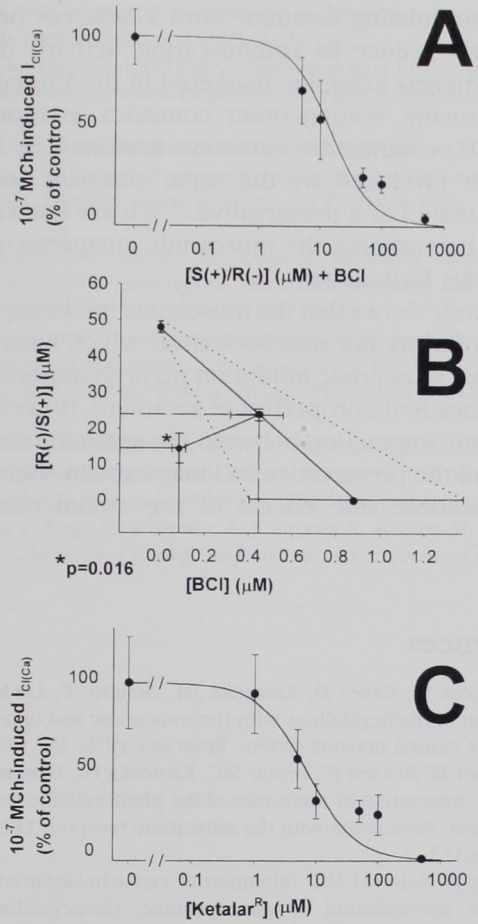

Fig. 4. The muscarinic inhibitory action of the racemic S(+)/R(−) ketamine/benzethonium chloride (BCl) mixture and Ketalar. Oocytes expressing the m1 muscarinic receptor were voltage clamped at −70 mV, and inward Cl^- current was recorded in response to 10^{-7} M acetyl-β-methylcholine. At least five oocytes were used to determine each data point. (*A*) The concentration-inhibition relation of muscarinic inhibitory action of racemic S(+)/R(−) ketamine with BCl in a concentration equivalent to that present in Ketalar. The calculated median inhibitory concentration is 15 ± 2 μM, and the Hill coefficient is 0.6. (*B*) Isobolographic analysis of the interaction between equimolar S(+)/R(−) ketamine and BCl. The interaction is synergistic, and R is 2.5. *P = 0.016. (*C*) The concentration-inhibition relation of the muscarinic inhibitory action of Ketalar. The calculated median inhibitory concentration is 7.2 ± 3.8 μM, and the Hill coefficient is 0.7.

than 0.1 mg/ml, a greater degree of synergism would be present.

Discussion

The main findings from this study are 1) the inhibition of muscarinic signaling by ketamine is not stereoselective, 2) the isomers act synergistically in their effect on muscarinic signaling, 3) BCl is a muscarinic antagonist, and 4) BCl acts synergistically with ketamine in its effect on muscarinic signaling.

Muscarinic signaling has been a subject of significant interest recently, particularly as it has become clear that muscarinic pathways in the central nervous systems are relevant to several anesthetic states, such as consciousness, learning, and pain perception.[9] In addition, the cloning of the muscarinic receptors[19] and continuing advances in molecular biology have facilitated the study of muscarinic signaling pathways. All five subtypes of muscarinic receptors are represented in the brain and usually in mixed populations.[20] Because different subtypes might have different anesthetic sensitivities and because no completely selective agonists and antagonists are available, direct study of brain tissue is difficult. For this reason, we chose to express one specific subtype of receptor in an environment that is easily studied. Choice of subtype was guided by brain distribution: the m1 receptor represents 34–40% of muscarinic receptor protein in cortex and 47% in hippocampus, making it the most prominent subtype in these areas.[20] Because of the relevance of these areas to anesthetic action, we chose to study the m1 receptor. Our findings, therefore, do not preclude the possibility that ketamine might act in a stereoselective manner at these other receptors.

Muscarinic receptors have been expressed frequently in the *Xenopus* oocyte model system by ourselves[6,13] and by other investigators,[19,21] and it is well established that they function similarly in oocytes and in native cells. Although the receptors are expressed at a lower temperature than they are normally exposed to, this has not been shown to result in significant changes in kinetics or function. Therefore, we are confident that our results can be extrapolated to cells with endogenously expressed muscarinic receptors. Obviously, they cannot be extrapolated to the other muscarinic receptor subtypes.

Our finding that the muscarinic antagonistic properties of ketamine are not stereoselective is important because it makes muscarinic inhibition unlikely to be essential in ketamine's mode of anesthetic action, which is strongly stereoselective: S(+) ketamine is approximately three or four times as potent an anesthetic as R(−) ketamine, whereas R(−) ketamine exhibits more psychic side effects.[10,11]

Two other implications follow from the lack of stereospecificity observed in the present study. First, it suggests a benefit of using the S(+) isomer as a clinical

anesthetic: when used in equipotent concentrations, it would exhibit less muscarinic inhibition than the R(−) isomer does. Second, our findings suggest that (nonstereoselective) muscarinic inhibition by ketamine is mediated by a different part of the molecular structure than (stereoselective) anesthetic action is. If muscarinic inhibition is not essential to the anesthetic action of ketamine, it might be possible to develop compounds that retain ketamine's anesthetic activity yet lack muscarinic inhibitory action. The observed Hill coefficients indicate that the interaction is unlikely to be cooperative in nature.

Whereas ketamine's primary mode of anesthetic action is considered to be competitive antagonism at the N-methyl-D-aspartate receptor,[7,8] this does not preclude the relevance of secondary activities. A recent report suggests that the direct cardiac depressant effects of ketamine might be due to interaction with cardiac Ca^{2+} channels.[22] The compound was shown to decrease contractile force and transsarcolemmal Ca^{2+} current. As in our study, the S(+) and R(−) isomers were of similar potency. However, this issue cannot be considered resolved because other investigators found the S(+) isomer to induce less cardiac depression than the R(−) isomer, a result attributed to increased availability of catecholamines.[23] Suppression of neutrophil function by ketamine has been shown to be nonstereoselective.[24] It appears, therefore, that at least some secondary effects of ketamine can result from nonstereoselective interactions.

Our finding that BCl is a muscarinic antagonist by itself was surprising. To our knowledge, an action on muscarinic signaling for this compound has not been suggested before. The compound has been used primarily as a topical antiseptic or anti-infective agent, and also as a preservative, as with Ketalar. There are, however, similarities in chemical structure between BCl and several synthetic antimuscarinic agents, such as eucatropine, tridihexetyl chloride, and tropicamide: All consist of a modified ring structure separated by a carbon chain of variable length from a modified choline group. Hence, in retrospect, it is not surprising that BCl would have significant antimuscarinic activity. Of interest is the finding that it acts synergistically with ketamine. In the absence of a synergistic interaction, the concentration of BCl in Ketalar would be too low to be clinically relevant as an antimuscarinic compound. However, our data indicate that the presence of such low concentrations of BCl potentiates the antimuscarinic action of ketamine by approximately three times. This suggests that reformulating ketamine with a different preservative might reduce its antimuscarinic activity. Interestingly, whereas ketamine marketed in the United States and Germany, among other countries, is formulated with BCl, commercial ketamine marketed in France, although produced by the same company, contains chlorobutanol as a preservative.[25] We are not aware of studies determining the muscarinic antagonist properties of this formulation.

Our study shows that the muscarinic inhibitory effects of ketamine are not stereoselective, which makes it unlikely that muscarinic inhibition plays an important role in the anesthetic properties of ketamine. However, the synergistic interaction between the ketamine stereoisomers and the preservative BCl may explain some of the antimuscarinic side effects of the commercial compound.

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