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Ketamine enantiomers and acetylcholinesterase

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Ketamine hydrochloride (2-(*o*-chlorophenyl)-2-methylaminocyclohexanone hydrochloride) is a drug with sedative, analgesic and anaesthetic properties [1]. As used clinically, ketamine is a racemic mixture, of equal concentrations of dextrorotatory (+)- and levorotatory (−)-isomers. With the aim of developing drugs with higher potency and fewer side effects, there is currently a great interest in studying the action of isolated isomers of pharmacologically active substances with two (or more) isomers. Studies on the enantiomers of ketamine in animals [2–4] and in humans, both healthy volunteers [5, 6] and patients [7], reveal a consistent picture: the (+)-isomer is more potent (approximately three times), has a more favourable therapeutic index and gives rise to less psychic emergence reactions in the postanaesthetic period than the (−)-form.

Ketamine has many effects, which have been studied at the cellular and molecular levels. The most prominent action is to antagonize, in a non-competitive way, *N*-methyl-D-aspartate receptor responses [8, 9]. Some of the molecular actions have been studied with isolated enantiomers and potency differences have been found, but in no case exceeding a factor of five, i.e. in the same range as found *in vivo*. In almost all cases, e.g. displacement of phencyclidine-binding to brain membranes [10], the (+)-isomer was found to be the most potent isomer. An exception is high affinity transport of serotonin, which was somewhat more inhibited by the (−)-isomer than by the (+)-form [11].

Our interest in ketamine is coupled to the action of anticholinesterase compounds, primarily nerve agents. One could, in the military context, expect the occurrence of "mixed casualties", i.e. patients intoxicated by nerve agent but who also have conventional injuries, prompting the use of an anaesthetic. The anaesthetic of choice must not aggravate the intoxication or counteract the action of medical treatment against the intoxication (atropine and an oxime). In an initial study on pigs, we noticed that ketamine-anaesthesized animals tolerated nerve agent better than did pentobarbital-anaesthesized animals [12], an effect most probably due to the ionic channel blocking mentioned above. We also found that ketamine was beneficial in intoxicated and antidote (atropine and HI-6)-treated guinea-pigs [13]. At the molecular level, we investigated ketamine effects on purified acetylcholinesterase (EC 3.1.1.7). Inhibition constants were determined, both for solubilized and, as ketamine has direct membrane-perturbing properties, on enzyme reconstituted into liposomes [14]. We showed that ketamine protects the enzyme from irreversible inhibition by an organophosphorus inhibitor, sarin (isopropyl methylphosphonofluoridate), and interfered with reactions of importance for the therapeutic counter-measures against organophosphorus intoxication, i.e. dealkylation ("aging") and reactivation of sarin-inhibited acetylcholinesterase [15]. In the present study we repeated these experiments, with ketamine enantiomers.

Materials and Methods

Ketamine HCl racemate and (+)-ketamine HCl were gifts from Warner Lambert Scandinavia AB/Parke-Davis, while (−)-ketamine HCl was kindly donated by Dr A. Maurset, University of Oslo, Norway. Dr J. Clement, DRES, Canada, kindly gave us HI-6 dichloride. Sarin was synthesized in the chemistry division of this institute. Acetylcholinesterase was purified from bovine brain [14] and had a specific activity of 30 μkat/mg. The inhibition constants for the two isomers against acetylcholinesterase were determined as described recently [14] and the reaction constants for sarin inhibition, reactivation and aging according to [15].

Results and Discussion

The two enantiomers of ketamine are both inhibitory towards acetylcholinesterase and the inhibition is of a mixed type (Table 1) thus affecting both the binding of the substrate and the velocity for its hydrolysis. We found that the (−)-form was slightly more potent, having a lower K_i,

Table 1. Inhibition constants for ketamine and its isomers towards acetylcholinesterase

	(±)-Ketamine	(−)-Ketamine	(+)-Ketamine
Acetylcholinesterase in Triton X-100			
K_i (mM)	0.42 (0.05)	0.37 (0.03)	0.45 (0.005)*
K_i' (mM)	3.5 (0.4)	2.5 (0.2)	4.2 (0.2)‡
Acetylcholinesterase in liposomes			
K_i (mM)	0.34 (0.05)	0.26 (0.02)	0.35 (0.02)‡
K_i' (mM)	3.2 (0.3)	2.7 (0.2)	4.0 (0.8)†

The data are given as mean values with standard deviations in parenthesis, $N = 4$ for all experiments. The difference between the values for (−)-ketamine and (+)-ketamine was tested by Student's t-test. *$P < 0.1$, †$P < 0.01$ and ‡$P < 0.001$.

than the (+)-form. For the non-competitive component of the inhibition, K_i', we also found a lower value for the (−)-form and the relative difference between the constants was greater. The inhibition constants were also determined towards acetylcholinesterase reconstituted in liposomes. The relative potencies of the two isomers were comparable to the values for the solubilized enzyme. As we have shown [14], the membrane-bound enzyme is more susceptible to ketamine inhibition.

We also studied how the isomers protect the enzyme against inhibition by an irreversible inhibitor. For this aim, we determined the bimolecular reaction constant, k_i, for the organophosphorus inhibitor sarin in the presence of different concentrations of each enantiomer of ketamine. As could be expected from the inhibition constants in Table 1, the (−)-isomer was more efficient than the (+)-isomer in preventing the irreversible inhibition by sarin. The protection could, for both isomers, be described by the most simple kinetics, i.e. $k_{obs} = k_i/(1 + [\text{ketamine}]/K_i)$. A 50% reduction of k_i is thus obtained when the concentration of ketamine equals its K_i value. We did not find this simple relationship when we studied ketamine racemate [15].

Finally we also looked at the effects of the enantiomers on two reactions of the phosphonylated enzyme: reactivation and "aging". The reactivation efficacy is expressed as k_2/K_d. As shown in Table 2 both isomers of ketamine lowered the efficacy of the oxime HI-6 and, once again, (−)-ketamine was the most potent isomer. An interesting qualitative distinction was found when separating K_d (dissociation constant) and k_2 (reactivation rate constant). While the two isomers were comparable in their ability to modify the affinity for HI-6, only (−)-ketamine had an influence on reactivation rate, lowering it to about 70% of the control value.

A similar distinction was found in the aging reaction. Aging is dealkylation of the phosphonylated enzyme, a reaction autocatalysed by the enzyme. We have found previously that some ions can modulate the rate of aging, fluoride accelerating [16] and propidium and ketamine retarding [15, 17]. From Table 2 we can conclude that the retarding effect by ketamine is entirely due to the (−)-isomer. The depression is about 30% also for this rate constant.

We suggest that both isomers bind to a site at or near the active site of the phosphonylated enzyme and hinder the access of the oxime. The distinct feature of (−)-ketamine as regards the rate constants for aging and reactivation we would attribute to the binding to a peripheric site, probably the propidium binding site, resulting in a conformational change of the enzyme. It cannot be excluded that (+)-ketamine also binds to that site but without influence on the enzyme conformation.

As mentioned above we have noticed in animal experiments that ketamine relieves the symptoms, especially convulsions, caused by nerve agents. In antidote-treated animals we furthermore observed that the mortality was lower in the anaesthetized group than in the control group. We attribute these positive effects to the binding to NMDA-receptors and believe that this binding is much more important than the effects of ketamine directly on acetylcholinesterase-linked reactions such as reactivation by HI-6.

In summary, (−)-ketamine was found to be more potent than (+)-ketamine in all the studied reactions with acetylcholinesterase. In most cases the difference was small but for two rate constants the (−)-form was unique in having effects. Thus, the stereoselectivity of ketamine in this system is the opposite of most other systems studied.

Table 2. The effect of 5 mM ketamine enantiomers on kinetic parameters for reactivation by HI-6 and for aging of sarin-inhibited acetylcholinesterase

	Reactivation			Aging
	k_2/K_d ($10^3\,M^{-1}\,min^{-1}$)	k_2 (min^{-1})	K_d (μM)	k_a (hr^{-1})
Control	1.7 (0.1)	0.032 (0.007)	20 (5)	0.32 (0.03)
(−)-Ketamine	0.45 (0.04)‡	0.021 (0.008)*	49 (23)*	0.24 (0.02)†
(+)-Ketamine	0.75 (0.42)†	0.031 (0.012)	54 (34)*	0.32 (0.06)

The data are given as mean values with standard deviations in parentheses, $N = 3$ for all experiments. The difference between the control values and those obtained in the presence of ketamine was tested by Student's t-test, *$P < 0.1$, †$P < 0.05$ and ‡$P < 0.001$.

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β-Hydroxybutyrate: a urinary marker of imipenem induced nephrotoxicity in the cynomolgus monkey detected by high field ^{1}H NMR spectroscopy

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High field nuclear magnetic resonance (NMR) spectroscopy is finding increasing application in the analysis of biological fluids obtained during the investigation of drug metabolism or toxicity (reviewed in Refs 1–3). In particular Gartland *et al.* [4] have demonstrated the utility of proton (^{1}H) NMR of urine as a means of obtaining information on the site of toxic insult and the biochemical mode of action of a wide range of compounds exhibiting nephro-, hepato- and testicular toxicity. We have previously used ^{1}H NMR as a means of urinalysis for studies on the effects of the cephalosporin antibiotic cephaloridine [5, *] in order to monitor the onset and progress of nephrotoxicity. As part of further investigations on antibiotic nephrotoxicity we have used ^{1}H NMR for the qualitative analysis of urine samples obtained from cynomolgus monkeys dosed with the carbapenem antibiotic imipenem as described below.

Materials and Methods

Animals and treatments. Experimental work was conducted at Inveresk Research International (Musselburgh, U.K.). In this study three male and three female cynomolgus monkeys (*Macaca fascicularis*, Shamrock

* Murgatroyd LB, Pickford RJ, Smith IK and Wilson ID, submitted.