Differential Effects of Ketamine Isomers on Neuronal and Extraneuronal Catecholamine Uptake Mechanisms

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Contractile responses of isolated rabbit aortic strips to epinephrine and norepinephrine were potentiated in a dose-related manner by (+) ketamine but not by (-) ketamine (1.1 × 10^{-3} M – 3.7 × 10^{-4} M). Potentiation was blocked completely by pretreatment with the extraneuronal uptake inhibitor cortisol (83–138 μM) but was unaffected by the neuronal uptake inhibitor cocaine (29 μM). Responses of the rat anococcygeus muscle to these catecholamines were potentiated by both isomers, with (+) ketamine being more potent than its optical antipode. These effects were blocked completely in tissues from 6-hydroxydopamine sympathectomized animals. Results suggest that inhibition of extraneuronal uptake of catecholamines by racemic ketamine is due solely to an action of the (+) isomer, whereas both isomers appear capable of inhibiting neuronal uptake. (Key words: Anesthetics, intravenous; ketamine isomers. Sympathetic nervous system: catecholamines; uptake.)

Clinically available ketamine is a racemic mixture of its levo (-) and dextro (+) hydrochloride salts. (+) Ketamine is more potent than (-) ketamine in a variety of biological systems.1-4 This has led investigators to suggest a place for (+) ketamine as a more clinically useful anesthetic than either the racemic mixture or the (-) isomer.2,4,5

The demonstration that racemic ketamine can block both neuronal and extraneuronal catecholamine uptake sites at similar concentrations5 has prompted us to study the effects of ketamine isomers on catecholamine responses in different types of smooth muscle. The rabbit aorta has a sparse adrenergic innervation but has an avid extraneuronal uptake system7 that is inhibited by racemic ketamine.8 The rat anococcygeus muscle is a densely innervated tissue that has little or no ability to metabolize catecholamines extraneuronally.8 Racemic ketamine has been shown to potentiate catecholamine responses in this tissue by inhibition of neuronal uptake.6 We have examined the possibility that neuronal and extraneuronal catecholamine uptake inhibition by ketamine is stereospecific at one or both sites.

Materials and Methods

Helically cut strips of rabbit thoracic aorta were prepared according to the method of Furchgott10 and paired anococcygeus muscles as described by Gillespie.11

Tissues were suspended in organ baths containing a modified Krebs-Henseleit solution as described previously.6

Smooth muscle contractions were recorded auxotonically using Harvard12 smooth muscle transducers connected to a Rikadenki12 multichannel chart recorder. Chemical sympathectomy using 6-hydroxydopamine (6-OHDA) 250 mg/kg, intraperitoneally, 48 h prior to death was judged to be complete under previously outlined criteria.6

The effects of ketamine isomers on responses to catecholamines were examined initially by addition of either (+) ketamine or (-) ketamine (added either cumulatively or in single doses) to tissues that were precontracted to a steady plateau by low concentrations of (-) epinephrine or (-) norepinephrine, or by constructing cumulative dose-response curves, in the absence or presence (including a 15 min pretreatment) of either of the ketamine isomers at various dose levels. All results are expressed as a per cent of the maximal contractile response to added catecholamine.

Racemic ketamine (supplied by Bristol-Myers) was resolved according to the method of Hudyma.‡ The optical purity of the isomers was determined as described by Dale et al.12 and found to be no less than 95% (+)-ketamine HCl [melting point 258–259° C, [α]D^{23.5} = +90.43° (c 1.99 H₂O)] or (-)-ketamine HCl [melting point 258–260° C, [α]D^{23.5} = −92.02° (c 1.86 H₂O)].

Drugs used in this study were (-) arterenol and (-) epinephrine bitartrates, cortisol, tyramine HCl, and 6-OHDA (all from Sigma) and cocaine HCl (Mallinkrodt). Drug solutions were freshly prepared on the day of use and kept chilled on ice. Cortisol was dissolved in propylene glycol (10 mg/ml), catecholamines in 0.01 N HCl, and 6-OHDA in 0.001 N HCl.

‡ Hudyma TW, Holmes SW, Hooper IR: Splitting and isolating pharmacologically active isomers of racemic ketamine 2-(α-chlorophenyl)-2-(methyl-alino)-cylohexanone. Chemical Abstracts 75, 118119X, 1971
Fig. 1. Differential effects of ketamine isomers on catecholamine-contracted rabbit aorta. \( (+) \) Strip contracted by epinephrine (E) 30 nM and exposed to \( (+) \) ketamine (K) 50 \( \mu \)g/ml (110 \( \mu \)M). \( (-) \) Strip contracted by E and exposed to \( (-) \) K 50 \( \mu \)g/ml (110 \( \mu \)M). \( (\text{lower}) \) Strips from same aorta contracted by norepinephrine (NE) 30 nM and exposed to the K isomers. Drug additions are indicated by dots.

Data were compared at the EC\(_{50}\) level following regression analysis over the straight portion of the dose-response curves and then by comparison of these values using Student's \( t \) test.

**Results**

Initial studies demonstrated that \( (+) \) ketamine (3–100 \( \mu \)g/ml; 11–370 \( \mu \)M) potentiated responses to the catecholamines in rabbit aorta, whereas \( (-) \) ketamine at these concentrations did not. \( (+) \) Ketamine produced maximal potentiation at 30 \( \mu \)g/ml, and we have used this concentration throughout this study. Typical responses are shown in fig. 1.

Potentiation of catecholamine responses following 30 \( \mu \)g/ml \( (+) \) ketamine was completely abolished by the extraneuronal uptake inhibitor cortisol (83 \( \mu \)M) but not by cocaine (29 \( \mu \)M). \( (-) \) Ketamine had only a direct depressant action (fig. 2).

In the rat anococcygeus, by contrast, both isomers of ketamine potentiated catecholamine responses. Typical potentiation of norepinephrine responses of this tissue by \( (+) \) ketamine and \( (-) \) ketamine are shown in fig. 3.

Dose-response curves using both isomers of ketamine with epinephrine as the agonist were constructed and results are presented in figs. 4 and 5 and in table 1. In rabbit aorta, 10 \( \mu \)g/ml \( (+) \) ketamine shifted the epinephrine dose–response curve to the left 4.5-fold at the EC\(_{50}\) while the \( (-) \) isomer shifted the curve 2.3-fold (fig. 5 and table 1). Concentrations of ketamine isomers used in these studies, 30 \( \mu \)g/ml, did not produce maximal potentiation in the anococcygeus muscle but were chosen to be consistent with that dose which was maximally effective on rabbit aorta. In 6-OHDA pretreated tissues, neither ketamine isomer potentiated the response of the anococcygeus muscle.

**Discussion**

Racemic \( (\pm) \) ketamine has been reported to inhibit extraneuronal uptake,6,8 neuronal uptake,6,13 or both uptake processes,6,14 depending on the relative importance of the two uptake sites in the tissues examined.6 The results of the present study are consistent with the view that \( (+) \)
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Ketamine inhibits extraneuronal uptake of the catecholamines, while (-) ketamine was without effect and, therefore, that the (+) isomer is probably totally responsible for the effect of racemic ketamine on extraneuronal mechanisms in this tissue. In the rabbit aorta, specifically, much of the ability of cocaine to potentiate catecholamines is due to postsynaptic effects of the drug. Potentiation of catecholamine responses by the (+) isomer of ketamine following cocaine or desmethylimipramine pretreatment of aortic strips (unpublished observations), taken together with evidence from previous studies using racemic ketamine, indicate that (+) ketamine is responsible for inhibition of extraneuronal catecholamine uptake. (-) Ketamine has only direct depressant activity on aortic strips. Ketamine, therefore, appears to inhibit the extraneuronal uptake site in a stereospecific manner.

The effects of ketamine isomers on the peripheral neuroeffector systems examined in vitro in this study show that both isomers of ketamine inhibited neuronal uptake in the anococcygeus muscle. However, the (+) isomer was somewhat (approximately 2-fold) more potent than the (-) isomer, although the difference in potency reported in this study was less than that reported following the use of the same isomers on synaptosomal preparations.

In addition, the observation that the isomers differentially affected the two uptake sites at similar concentrations may help to explain the unique ability of racemic ketamine to block both uptake sites in peripheral tissue. These observations appear to clarify certain apparent discrepancies in the literature concerning these effects, which have been variously ascribed to its ability to block neuronal or extraneuronal catecholamine uptake.

Our results supply a possible rational for certain of the differential physiologic effects of ketamine isomers reported by others. For example, the cardiovascular activity of ketamine has been suggested to be the result of: 1) central stimulation of sympathetic outflow with 2) subsequent block of re-uptake of released catecholamines. In humans, anesthetic doses of the two iso-

![Figure 3](image-url)

**Figure 3.** Potentiating effects of ketamine isomers on norepinephrine-contracted anococcygeus muscles. (Upper) Muscle contracted by norepinephrine (NE) 100 nM and exposed to (-) ketamine (K) to achieve concentrations (µg/ml) of 1, 3, 10, 30, 100, and 300 (3.7, 11.3, 37.0, and 1100 µM) administered cumulatively. (Lower) Muscle contracted by NE as above and exposed to equivalent concentrations of (+) K. Tissues are paired muscles from the same animal.

![Figure 4](image-url)

**Figure 4.** Rabbit aorta: Effects of ketamine isomers on dose–response curves to epinephrine. (A) In the absence and presence of (+) ketamine (Ket) (10 and 30 µg/ml: 37 and 110 µM) or (-) Ket (30 µg/ml: 110 µM). (B) In the absence and presence of (+) Ket (30 µg/ml: 110 µM) following pretreatment with cortisol (30 µg/ml: 85 µM). Values are shown with their means ± SEM. NS = not significant P > 0.05.
mers of ketamine result in blood concentrations of about 1 and 3 µg/ml for the (+) and (-) isomer, respectively, and in rats, isomer concentrations reach 10–100 µg/ml. If a comparison of blood (10 µg/ml) with brain (100 µg/ml) concentrations of ketamine isoers attained in rats is made, and comparisons made to humans, it may be suggested that tissue concentrations in humans may reach somewhat higher concentrations than those reported in human blood. It would be difficult to compare tissue levels of ketamine isoers in man and animals to the concentrations used in the in vitro systems in this study. It is possible to suggest, however, that concentrations used in the present study (1–30 µg/ml) are certainly pharmacologically relevant to the in vivo situation (1–100 µg/ml). Even the highest concentrations routinely used in the present in vitro study are lower than concentrations attained in some of the in vivo situations outlined previously. These results further suggest that inhibition of the two catecholamine uptake sites, possibly following central stimulation of sympathetic mechanisms, plays a role in attaining increased circulating catecholamine levels during ketamine anesthesia.

**TABLE 1. Effect of Ketamine (Ket) Isomers on Catecholamine Responses of Rabbit Aorta and Rat Anococcygeus Muscle Following Various Treatments**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>Catecholamine EC₅₀ X 10⁻⁴ ± SEM(M)</th>
<th>Number of Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>Epinephrine control in presence of</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(+) Ket (10 µg/ml)</td>
<td>7.2 ± 1.6</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>(30 µg/ml)</td>
<td>2.1 ± 0.5*</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>(−) Ket (30 µg/ml)</td>
<td>1.2 ± 0.5*</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>cortisol (30 µg/ml)</td>
<td>4.6 ± 1.1†</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>(+) Ket (30 µg/ml) + cortisol (30 µg/ml)</td>
<td>2.8 ± 1.0*</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Norepinephrine control in presence of</td>
<td>1.8 ± 0.3*§</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>(+) Ket (30 µg/ml)</td>
<td>6.5 ± 2.9</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>(−) Ket (30 µg/ml)</td>
<td>1.8 ± 0.58*</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Anococcygeus</td>
<td>4.8 ± 1.30†</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Epinephrine control in presence of</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(+) Ket (30 µg/ml)</td>
<td>20.6 ± 8.4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>(−) Ket (30 µg/ml)</td>
<td>4.6 ± 0.03*</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Epinephrine + 6-OHDA in presence of</td>
<td>9.1 ± 0.02*‡</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>(+) Ket (30 µg/ml)</td>
<td>0.90 ± 0.18</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>(−) Ket (30 µg/ml)</td>
<td>0.86 ± 0.18‡</td>
<td>11</td>
</tr>
</tbody>
</table>

* Values significantly different from respective catecholamine controls, P < 0.05.
† (−) Ket produced no significant potentiation compared with catecholamine controls.
‡ (−) Ket produced significantly less potentiation than (+) ketamine.
§ (+) Ket produced no further significant potentiation in cortisol pretreated strips.
¶ (±) or (−) Ket produced no significant potentiation in 6-OHDA pretreated anococcygeus.

FIG. 5. Rat anococcygeus muscle: Effect of ketamine isoers on dose-response curves to epinephrine. (A) In the absence and presence of (+) ketamine (Ket) or (−) Ket (30 µg/ml: 110 µM). (B) On tissues from 6-OHDA-created animals. Values are shown with their means ± SEM.
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References