The Effect of Ketamine on Catecholamine Metabolism in the Isolated Perfused Rat Heart

David J. Miletich, Ph.D.,* Anthony D. Ivankovic, M.D.,† Ronald F. Albrecht, M.D.,‡ Behrooz Zahed, M.D.,§ Aitha A. Ilahi, M.D.¶

The effects of ketamine infusion on the uptake and release of norepinephrine and on formation of deaminated metabolites in the isolated, perfused rat heart were studied. Hearts were removed from animals during light ether anesthesia, transferred to a modified Langendorff perfusing apparatus, and perfused with Krebs-Ringer solution containing various doses of ketamine and 200 ng/ml of Dl-norepinephrine-14C. Norepinephrine uptake was determined after perfusion for 1, 2, 4, 6, and 20 minutes. At the end of each period the ketamine-treated hearts contained less Dl-norepinephrine-14C per gram of heart tissue than control hearts. The reduction was proportional to the dose of ketamine. On an equal-dose basis, ketamine was approximately 80 per cent as effective as cocaine in blocking the uptake of norepinephrine. Norepinephrine release was determined after perfusing hearts for 4 minutes with perfusate containing 200 ng/ml of Dl-norepinephrine-14C, then perfusing with solution containing ketamine but free of norepinephrine for 20 minutes. After 10 minutes of perfusion a decrease in myocardial Dl-norepinephrine began to appear, and at 20 minutes the decrease was significant. Ketamine infusion did not alter myocardial levels of intracellular deaminated metabolites of norepinephrine, indicating that the effects of ketamine are primarily membrane-related. The results of this study suggest that the sympathomimetic-like effects of ketamine seen in both man and animals may be due to inhibition of the uptake processes for endogenously released norepinephrine. (Key words: Ketamine; Norepinephrine; Cocaine; Isolated heart; Catecholamine metabolism.)

* Assistant Professor of Anesthesiology (Michael Reese).
† Associate Professor of Anesthesiology (Michael Reese).
‡ Professor of Anesthesiology; Chairman, Department of Anesthesiology (Michael Reese).
§ Associate Professor of Anesthesiology (Michael Reese).
¶ Resident (Michael Reese).

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Ketamine and related substances have been shown to increase heart rate and cardiac output, elevate arterial pressure, and enhance the effects of exogenously administered norepinephrine.¹-⁶ In man, ketamine has been shown to increase plasma norepinephrine concentrations after intravenous injection.⁷ Chang et al.⁸ demonstrated that the rise in systolic pressure seen in pithed rats after ketamine administration was abolished by prior reserpine treatment, and could be restored by norepinephrine infusion.

 Although the sympathomimetic-like action of ketamine has been adequately documented, no information concerning the direct effect of ketamine on the adrenergic, neurochemical transmitter, norepinephrine, has been reported. The purpose of this investigation was to study the effects of ketamine on the uptake, release, and deaminative metabolism of norepinephrine in the isolated, perfused, rat heart.

Methods

Male Sprague-Dawley rats weighing 200-250 g were used in all experiments. The heart was quickly removed from each animal during light ether anesthesia and washed in cold saline solution before perfusion. The hearts were perfused as described by Iversen except that a pressure transducer and a peristaltic bilateral roller pump were introduced into the perfusion line, as illustrated in figure 1. Each heart was perfused at a constant rate of 5 ml/min and at a perfusion pressure which averaged approximately 40 mm Hg. The basic perfusate medium was Krebs-Ringer solution with bicarbonate. In addition, each liter of perfusate contained 1 g glucose, 20 mg ascorbic acid, and 10 mg ethylenediaminetetraacetic acid disodium (EDTA). The perfusates were gas-saturated throughout the experiment with 95 per cent oxygen and 5 per cent carbon
dioxide. In each experiment the control and experimental hearts were first perfused for two minutes with physiologic Krebs-Ringer solution to wash the blood from the heart and to allow normal rhythmic beating to commence.

The control values for the uptake of DL-norepinephrine-\(^{14}\)C (Amersham/Searle, specific activity: 56 mCi/mmol) were determined by perfusing the hearts with Krebs-Ringer solution containing 200 ng/ml of norepinephrine-\(^{14}\)C for 1, 2, 4, 6, and 20 minutes. The hearts were then perfused for 90 seconds with norepinephrine-free perfusate to remove extracellular norepinephrine-\(^{14}\)C. After each period the hearts were homogenized, centrifuged at 500 x g, and 0.2-ml amounts of the supernatants were pipetted into vials containing 10 ml of phosphorethanol liquid scintillation-counting medium. The \(^{14}\)C content was analyzed by a liquid-scintillation spectrometer.

The radioactivity of the supernatants was converted to mols of norepinephrine by dividing the total radioactivity of the supernatants by the specific activity of DL-norepinephrine-\(^{14}\)C per mol. Uptake values for experimental hearts were determined in a similar fashion except that test doses of ketamine (Ketalar, Parke-Davis) or cocaine were added to the Krebs-Ringer perfusate.

The release of norepinephrine-\(^{14}\)C from control hearts was determined by first perfusing each heart with Krebs-Ringer solution containing 200 ng/ml of norepinephrine-\(^{14}\)C for 4 minutes, after which the hearts were perfused with normal Krebs-Ringer solution for periods of 10 and 20 minutes. The experimental hearts were perfused in the same manner except that ketamine (0.1 mg/ml) was added to the perfusate. At the end of each experiment the hearts were homogenized and 0.2 ml amounts
of the supernatant were analyzed for $^{14}$C content as described above.

Norepinephrine-$^{14}$C-deaminated metabolites were determined as described by Horst. After 10 minutes of perfusion with perfusate containing 200 ng/ml of norepinephrine, the hearts were homogenized in 10 ml of 0.4 N perchloric acid and centrifuged for 10 minutes at 500 x g. A 4-ml amount of each of the supernatants was prepared for deaminated metabolite extraction by acidification with 1 ml of 1 N HCl. The deaminated metabolites were extracted with 5 volumes of water-saturated ethyl acetate by shaking for 15 minutes in a separation flask. Samples of the organic phase were placed in counting vials containing phosphorothanol and analyzed for $^{14}$C content by liquid-scintillation counting. In addition, prior to deaminated metabolite extraction, a 0.2-ml sample was removed from each supernatant and analyzed for $^{14}$C content. The $^{14}$C activity of the latter fraction minus the $^{14}$C activity of the organic phase ($^{14}$C-deaminated metabolites) was considered to be mostly intact, nonmetabolized, norepinephrine-$^{14}$C. It was previously demonstrated that 95 per cent of the norepinephrine found in perfused hearts is intact and nonmetabolized. Aort Aortic pressures were continuously monitored as illustrated in figure 1 with a Statham pressure transducer. Heart rates for each heart were determined from the pressure recordings. Effluent volumes were collected and measured at the end of each experiment.

Results

The effects of perfusing hearts with perfusates containing ketamine in doses of 0.1 mg, 0.1 mg $\times 10^{-2}$, 0.1 mg $\times 10^{-3}$, and 0.1 mg $\times 10^{-3}$ per ml on the uptake of norepinephrine-$^{14}$C are represented in figure 2. The hearts were perfused at a rate of 5 ml/min for 6 minutes so that the total amount of ketamine infused were 3.0 mg, 0.3 mg, and 0.03 mg, and 0.003 mg, respectively. All concentrations of ketamine examined significantly reduced the uptake of norepinephrine-$^{14}$C by the heart. The degrees of reduction appeared proportional to the concentrations employed.

Table 1. The Effect of Ketamine (0.1 mg/ml) Infusion on Norepinephrine Metabolism (n = 6)*

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>b/a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>849.5 ± 308</td>
<td>17.9 ± 3.2</td>
<td>0.021</td>
</tr>
<tr>
<td>Ketamine</td>
<td>422.8 ± 105</td>
<td>10.8 ± 1.6</td>
<td>0.025</td>
</tr>
</tbody>
</table>

* a = ng norepinephrine-$^{14}$C/g heart.

b = ng deaminated-$^{14}$C metabolites/g heart.

$b/a$ = norepinephrine-$^{14}$C: deaminated-$^{14}$C metabolite ratio.

The reduced uptake did not appear to be the result of changes in intramyocardial hemodynamics since, compared with control values, neither perfusion pressure nor effluent volumes were affected by ketamine (fig. 3). Control heart rates were 210 ± 30 beats/min at the end of the 6 minutes of perfusion. Compared with control heart rates, heart rates were unaffected by any of the ketamine dosages throughout the 6-minute period. However, in subsequent experimentation it was found that ketamine infusion in doses larger than 0.01 mg/ml generally reduced the average heart rate when the perfusion time was extended beyond 6 minutes, indicating a time-dependent cumulative effect.

In figure 4 can be seen the effect of ketamine (0.1 mg/ml) on the uptake of norepinephrine-$^{14}$C during 20 minutes of perfusion. The ability of ketamine to prevent norepinephrine-$^{14}$C uptake was seen as early as one minute after the commencement of perfusion and persisted throughout the 20-minute perfusion period. Compared with 20-minute control values, effluent volumes and perfusion pressures were unchanged by ketamine treatment. However, heart rate was reduced in the ketamine-treated hearts, as previously noted for extended perfusion periods. Control heart rates were 185 ± 31 beats/min after 20 minutes of perfusion; ketamine-infused hearts had rates of 114 ± 22/min.

Figure 5 compares the effects of equal doses (0.01 mg/ml) of ketamine and cocaine on the 6-minute uptake of norepinephrine-$^{14}$C by the heart. On an equal-dose basis, cocaine would appear to be 21 per cent more effective than ketamine in preventing uptake. However, ex-
pressed on a mol-for-mol basis, ketamine and cocaine appear to be approximately equal in ability to reduce the uptake of norepinephrine-$^{14}$C. Cocaine reduced the average heart rate to $140$ $\pm$ $10$ beats/min by the end of the perfusion period. As previously observed, ketamine at this concentration had no apparent effect on heart rate after 6 minutes of perfusion.

The effect of ketamine (0.1 mg/ml) infusion on the release of norepinephrine-$^{14}$C was not as striking as its effect on the uptake of norepinephrine-$^{14}$C (Fig. 6). After 10 minutes

![Graph showing effect of ketamine on norepinephrine uptake](image1)

**Fig. 2.** The effect of ketamine on the 6-minute uptake of norepinephrine-$^{14}$C (200 ng/ml) by the heart. Left to right: control values; $0.1$ mg $\times 10^{-3}$, $0.1$ mg $\times 10^{-2}$, $0.1$ mg $\times 10^{-1}$, and $0.1$ mg ketamine per ml of perfusate. Each column represents the mean $\pm$ SE. All doses of ketamine significantly reduced uptake ($P < 0.01$, n = 6).

![Graph showing effects of ketamine on aortic pressure](image2)

**Fig. 3.** The effects of ketamine (0.1 mg/ml of perfusate) on aortic pressure and effluent volumes throughout a 6-minute perfusion period. Each column represents the average value $\pm$ the range (n = 6). No significant difference was seen in either.
of perfusion no significant decrease in the myocardial content of norepinephrine-$^{14}$C was observed. It is possible that such a decrease occurred but was obscured by the washout of vascular and interstitial norepinephrine-$^{14}$C. However, compared with control concentrations, after 20 minutes of perfusion a significant decrease in the myocardial concentration of norepinephrine-$^{14}$C was apparent. These observations suggest that the primary effect of ketamine in this experiment was an effect on the reuptake process, rather than an effect on the release of norepinephrine.

Table 1 shows the effect of ketamine infusion (0.1 mg/ml) on the ratio of deaminated $^{14}$C metabolites to intact norepinephrine-$^{14}$C in the heart after 10 minutes of perfusion. No significant difference between control and experimental ratios was found. It appears from these data that ketamine has no effect on the intracellular deamination of norepinephrine.

**Discussion**

Radiolabelled norepinephrine has been demonstrated to provide a convenient, reliable method for studying the effects of various agents on the metabolism of norepinephrine. Most of the infused radioactive norepinephrine retained by cardiac tissues predominately enters the cardiac sympathetic neurons, mixes with endogenous norepinephrine, and is subsequently metabolized in much the same fashion as the endogenous norepinephrine.

In this study the inclusion of ketamine in the perfusing medium reduced the myocardial content of norepinephrine-$^{14}$C in the isolated rat heart (fig. 2). The reduction was proportional to the dose of ketamine used and related to the sequence in which norepinephrine-$^{11}$C and ketamine were perfused. When ketamine and norepinephrine-$^{14}$C were perfused simultaneously, decreased myocardial uptake of norepinephrine-$^{14}$C was observed within a minute after the initiation of perfusion and persisted for at least 20 minutes (fig. 4). However, when the heart was first perfused with norepinephrine-$^{14}$C and then perfused with ketamine, the effect of ketamine in reducing the myocardial content of norepinephrine-$^{14}$C took longer than 10 minutes (fig. 6). These ob-
Fig. 5. The effects of ketamine (0.01 mg/ml and cocaine (0.01 mg/ml) on the 6-minute uptake of norepinephrine-14C (200 ng/ml) by the heart. Both ketamine and cocaine significantly reduced the uptake of norepinephrine-14C. Each column represents the mean ± SE (P < 0.01, n = 6).

Observations suggest that: 1) ketamine blocks the direct uptake of norepinephrine by the sympathetic neurons in the heart, and 2) if norepinephrine is permitted to enter the neuronal cells first, the effect of ketamine is then dependent upon the subsequent release and reuptake processes.

It is unlikely that ketamine reduced norepinephrine-14C uptake through an action on heart rate or coronary circulation. Ketamine did not change the effluent volume or heart rate during the 6-minute period of perfusion (fig. 3).

In addition to blocking the uptake of norepinephrine, ketamine could also have increased the release of norepinephrine by disrupting the intracellular storage mechanisms. Disruption of storage processes also results in elevated levels of intracellular deaminated metabolites due to the action of the cytoplasmic regulating enzyme monoamine oxidase. In this study, after 10 minutes of perfusion ketamine had no effect on the ratio of intracellular deaminated norepinephrine to intact norepinephrine as compared with control ratios (table 1). This may indicate that ketamine neither disrupted the intracellular storage mechanism nor caused an increase in release of norepinephrine during a perfusion period in which ketamine significantly blocked the uptake of norepinephrine-14C.

Previous reports have shown that phenycyclidine, a substance closely related to ketamine, produces sympathetic effects similar to those of cocaine. It was observed in this experi-

Fig. 6. The effects of ketamine (0.1 mg/ml) infusion of the 10- and 20-minute release of norepinephrine-14C. After 20 minutes of perfusion, ketamine had significantly reduced the myocardial content of norepinephrine-14C in the heart (P < 0.01). Each column represents the mean ± SE (n = 6).
ment that cocaine and ketamine were both effective in preventing norepinephrine uptake (fig. 5). These observations suggest that the qualitative similarities of the effects of ketamine-like substances and cocaine on the sympathetic system may be due to a common mode of action: initiation and potentiation of sympathetic activity by blocking the reuptake of norepinephrine at the sympathetic neuron.

It is difficult to assess the relevance of our findings to the clinical use of ketamine. Steady-state dosages of ketamine used during perfusion experiments cannot readily be compared with the bolus injection employed in the clinic. However, Hodshon et al., 14 utilizing a gas chromatographic assay procedure, found that plasma levels of ketamine in three patients were 5.8, 6.3, and 5.9 μg/ml 5 minutes after injection. Each of these patients received a 4-mg/kg dose. Based upon these findings, our observation that 0.1 μg/ml of perfusate was effective in blocking the uptake of norepinephrine could have clinical significance.

An additional difficulty in the interpretation of data from this study is that, unlike sympathomimetic amines, ketamine has been shown to depress the myocardium 15 and central nervous system, 16 and to reduce the frequency of baroreceptor responses. 17 From such evidence, it seems that the total effect of ketamine on the cardiovascular system is a manifestation of the balance between its apparent physiologic depressant and stimulatory effects.

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References

2. Kreuscher H, Gauch H: Die wirkung des phenecyclidinderivatives ketamine (CI-581) auf
das kardiovasculaure system des menschen. Anesthesiologie 16:229–233, 1967