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# THE EFFECT OF KETAMINE HYDROCHLORIDE, A NON-BARBITURATE PARENTERAL ANAESTHETIC ON OXIDATIVE PHOSPHORYLATION IN RAT LIVER MITOCHONDRIA

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**Abstract**—The effects of ketamine hydrochloride, a non-barbiturate parenteral anaesthetic on mitochondrial reactions are described. At low concentrations (5–150 μM) ketamine-HCl was found to inhibit the $NAD^+$-linked oxidation of glutamate plus malate in rat hepatic mitochondria. When succinate was the substrate ketamine-HCl was found to act as an uncoupling agent, maximum uncoupling occurring at a concentration of 50 μM. Ketamine-HCl was observed to release respiratory control, stimulate state 4 respiration, release state 3 respiration inhibited by oligomycin and to stimulate micochondrial ATPase. It was concluded that the second effect was due to an uncoupling action.

## INTRODUCTION

Ketamine-HCl [2-(-*O*-chlorophenyl)-2-(methylamino) cyclohexanone hydrochloride] is a short acting cataleptic analgesic and anaesthetic agent devoid of sedative or hypnotic properties, distinguishing it from other commonly used intravenous barbiturates [1–5].

Many anaesthetics [6] and steroids [7–9] have been shown to impair mitochondrial function, which has led to the suggestion that the maintenance of anaesthesia may be due to the depression of mitochondrial ATP synthesis [6], followed by the inhibition of the energy supply necessary to maintain impulse conduction [10].

As part of our investigation into the mode of action of ketamine-HCl we have tested the effects of this compound on mitochondrial reactions using mitochondria prepared from rat hepatic tissues.

## MATERIALS AND METHODS

*Oxygen consumption.* Oxygen consumption was measured polarographically, using an oxygen electrode (Rank Bros., Bottishman, U.K.) [11].

*ATPase.* ATP hydrolysis was measured by the method of Beechey [12], phosphate release being determined by the method of Fiske and Subbarow [13].

*NADH-ferricyanide reductase.* Ferricyanide reduction was measured spectrophotometrically at 420 nm by the method of Esmail *et al.* [8].

*Mitochondria.* Tightly coupled rat hepatic mitochondrial preparations were obtained by the method of Chappell and Hansford [14].

*Protein.* Protein was determined by the biuret method [15].

Specific reaction conditions are given in the legends to the figures and tables.

*Materials.* Ketamine hydrochloride (ketalar) was obtained from Parke-Davis Co. (Pontypool, U.K.) and was dissolved in distilled water. Oligomycin (Sigma Chemical Co., London, U.K.) and 2,4-dinitrophenol (British Drug House, Poole, U.K.) were dissolved in dimethylformamide.

## RESULTS

### *$NAD^+$-linked oxidation of glutamate plus malate*

Table 1 shows the effect of ketamine-HCl on the $NAD^+$-linked oxidation of glutamate plus malate in rat hepatic mitochondria. State 3 respiration (i.e. ADP present with substrate and oxygen in excess) was inhibited between 15 and 80 per cent by low concentrations of ketamine-HCl (5–150 μM) when glutamate (5 mM) plus malate (5 mM) were used as substrates. State 4 respiration (i.e. ADP absent, but substrate and oxygen in excess) was unaffected. The inhibition of state 3 respiration was not released by the addition of an uncoupling concentration of 2,4-dinitrophenol [DNP] (10 μM).

Using both ketamine-HCl and rotenone, a well known inhibitor of mitochondrial $NAD^+$-linked oxidations, mitochondrial difference spectra were obtained. Both compounds gave similar spectra. In the presence of these inhibitors cytochrome reduction was not achieved indicating their possible site of action to be on the substrate side of cytochrome *b*.

### *Ferricyanide reduction*

Table 2 shows the effects of ketamine-HCl and two NADH dehydrogenase inhibitors, rotenone and *p*-hydroxymercuribenzoate, on ferricyanide reduction in rat hepatic mitochondria. Both ketamine-HCl and rotenone failed to inhibit the reaction; in fact a variable stimulation of between 9 and 36 per cent was observed. *p*-Hydroxymercuribenzoate, on the other hand, gave a marked inhibition (51 per cent) of ferricyanide reduction. These results indicate that low concentrations of ketamine-HCl interact with

Table 1. The effect of ketamine-HCl on $NAD^+$-linked substrate and succinate oxidations

| Ketamine-HCl μM | Oxygen consumption (ngatom $min^{-1}$ $mg^{-1}$ mitochondrial protein) | | | |
|---|---|---|---|---|
| | Glutamate plus Malate | | Succinate | |
| | State 3 | State 4 | State 3 | State 4 |
| Control | 58.0 ± 0.8 | 12.3 ± 0.5 | 39.4 ± 2.0 | 22.5 ± 1.5 |
| 5 | 50.1 ± 1.4 | 12.3 ± 0.8 | 41.7 ± 1.0 | 34.5 ± 1.0 |
| 10 | 40.7 ± 0.8 | 12.3 ± 0.5 | 44.6 ± 1.7 | 52.5 ± 1.0 |
| 30 | 22.4 ± 1.0 | 12.3 ± 1.0 | 53.0 ± 1.0 | 53.4 ± 1.0 |
| 50 | 16.5 ± 1.5 | 12.3 ± 0.8 | 65.0 ± 1.0 | 65.0 ± 1.0 |
| 100 | 12.3 ± 0.8 | 12.3 ± 1.0 | 39.5 ± 1.5 | 39.5 ± 0.5 |
| 150 | 12.3 ± 1.0 | 12.3 ± 1.0 | 15.3 ± 1.0 | 15.3 ± 1.5 |

The reaction chamber of the oxygen electrode contained 675 μmol sucrose, 9.2 μmol Tris-HCl buffer, pH 7.4, 10 μmol potassium phosphate buffer, pH 7.4, and either 15 μmol sodium glutamate plus 15 μmol soduim malate or 15 μmol sodium succinate. The reactions were initiated by the addition of rat hepatic mitochondria (5 mg protein) to the electrode chamber, followed 2 min later by 0.5 μmol ADP. When present ketamine-HCl was added at the concentrations shown, at the start of the experiment. The temperature was 30° and the reaction volume was 3 ml. The results are the means of five experiments ± S.E.M.

the NADH dehydrogenase enzyme system, at or near, the rotenone sensitive site.

*Succinate oxidation*

A different pattern was obtained when glutamate and malate were replaced by succinate (5 mM). Both state 3 and state 4 respirations were stimulated by low concentrations of ketamine-HCl (5–100 μM). Maximum stimulation occurred at a ketamine-HCl concentration of 50 μM. (Table 1). This stimulation of both types of respiration was followed by a progressive inhibition until the rate of state 3 respiration equalled that of state 4. At a ketamine-HCl concentration of 160 μM the percentage inhibiton of state 3 and state 4 respiration was found to be 66 and 40 per cent respectively. The stimulation of state 4 respiration was similar to that obtained with the uncoupling agent DNP (10 μM), however DNP proved to be considerably more potent. Ketamine-HCl (50 μM), like DNP (10 μM) was found to release oligomycin (1 μg) inhibited state 3 respiration Fig. 1. These results with succinate indicated that ketamine-HCl [13] was acting as an uncoupling agent.

*ATP Hydrolysis*

Table 3 shows that ketamine-HCl elicited a 3-fold stimulation of the ATPase activity. Maximum stimulation occurred at a ketamine-HCl concentration of 50 μM (0.09 μmol inorganic phosphate released $min^{-1}$ $mg^{-1}$ mitochondrial protein); further increases in concentration resulted in a concentration dependent inhibition of the stimulated reaction. Similar results were obtained with DNP, with maximum

Table 2. The effect of ketamine-HCl on ferricyanide reduction in rat hepatic mitochondria

| Additions | Ferricyanide reduced (μmol $min^{-1}$ $mg^{-1}$ mitochondrial protein) |
|---|---|
| Control | 0.55 ± 0.02 |
| Ketamine-HCl (75 μM) | 0.60 ± 0.05 |
| Ketamine-HCl (150 μM) | 0.56 ± 0.05 |
| Ketamine-HCl (300 μM) | 0.70 ± 0.03 |
| Ketamine-HCl (600 μM) | 0.72 ± 0.05 |
| Rotenone (15 nmol) | 0.75 ± 0.05 |
| *p*-Hydroxymercuribenzoate (1 μmol) | 0.27 ± 0.02 |

The enzyme activity was measured spectrophotometrically by following the reduction of ferricyanide at 420 nm. Using a Pye Unicam S.P. 600 u.v. spectrophotometer. Blank and experimental cuvettes of cm light path contained 675 μmol sucrose, 30 μmol potassium phosphate buffer, pH 7.4, 2 μmol of potassium ferricyanide, 10 μg antimycin A, 500 μg sodium deoxycholate and rat hepatic mitochondria (2 mg protein). The reaction was initiated by the addition of 0.5 μmol NADH to the experimental cuvette. The temperature was 30° and the final volume was 3 ml. Inhibitors were added at the concentrations shown, at the start of the reaction. The results are the means of five experiments ± S.E.M.

Table 3. The effect of ketamine-HCl on rat hepatic mitochondrial ATPase

| Additions | ATPase (nmol inorganic phosphate released $min^{-1}$ $mg^{-1}$ mitochondrial protein) |
|---|---|
| Control | 25 ± 3 |
| Ketamine-HCl (10 μM) | 60 ± 5 |
| Ketamine-HCl (50 μM) | 93 ± 7 |
| Ketamine-HCl (100 μM) | 74 ± 5 |
| Ketamine-HCl (150 μM) | 58 ± 5 |
| Ketamine-HCl (10 μM) plus oligomycin (1 μM) | 14 ± 2 |
| 2, 4-Dinitrophenol (10 μM) | 130 ± 10 |
| 2, 4-Dinitrophenol (50 μM) | 63 ± 5 |

At zero time 0.1 ml of mitochondrial suspension (1 mg protein) was added to 0.9 ml reaction medium, which contained 125 μmol sucrose, 63 μmol Tris-HCl buffer, pH 7.4 and 25 μmol ATP. The reaction was maintained at 30° for 10 min when it was terminated by the addition of 0.1 ml 40 per cent (w/v) trichloroacetic acid. The inorganic phosphate liberated was estimated by the method of Fiske and Subbarow [13]. The results are the means of five experiments ± S.E.M.

stimulation occurring at a concentration of 10 μM. Table 3 also shows that the energy transfer inhibitor oligomycin (1 μg) prevented the stimulation of the ATPase produced in the control experiments by ketamine-HCl (10 μM).

## DISCUSSION

Many anaesthetic agents have been shown to interfere with mitochondrial function [6, 16, 17] and our present studies show that ketamine-HCl can be included in the same category.

At low concentrations ketamine-HCl inhibited the NAD$^+$-linked oxidation of glutamate plus malate; however it failed to prevent the reduction of ferricyanide, whole showing the ability to inhibit cytochrome reduction in a similar manner to rotenone. These findings suggest that its site of action may, like other anaesthetic agents [8] lie between the NADH dehydrogenase flavoprotein and cytochrome *b*, at a locus that may be either identical to or close to the rotenone sensitive site.

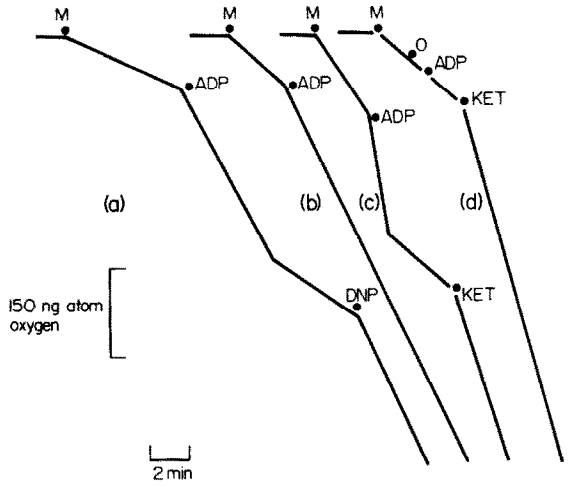


Fig. 1. The effect of ketamine-HCl on succinate oxidation in rat hepatic mitochondria. The experimental conditions were the same as described in the legend to Table 1, with 15 μmol sodium succinate acting as the substrate in all cases. Additions were made as follows; M, rat hepatic mitochondria (5 mg protein); ADP, 0.5 μmol ADP; DNP, 30 nmol 2, 4-dinitrophenol; KET, 150 nmol ketamine-HCl; O, 1 μg oligomycin. (a) Control showing effect of 2, 4-dinitrophenol (b) loss of respiratory control in the presence of 50 μM ketamine-HCl; (c) stimulation of state 4 respiration by ketamine-HCl; (d) release of oligomycin inhibited state 3 respiration by ketamine-HCl.

When succinate was used as the substrate ketamine-HCl only inhibited the respiration in the 60–160 μM concentration range. The maximum inhibition obtained was 66 per cent for state 3 and 40 per cent for state 4 respiration. In this concentration range ketamine-HCl also caused loss of respiratory control, indicating that it has the ability to uncouple oxidative phosphorylation. This was further supported by the findings that ketamine-HCl: (a) stimulated state 4 oxidation of succinate; (b) relieved oligomycin inhibited state 3 respiration; (c) stimulated mitochondrial ATPase in a concentration dependent manner; and (d) ATPase stimulation being sensitive to low concentrations of oligomycin (1 μg). Similar results have been obtained with other anaesthetic agents [6] and various steroids [8, 18].

The results indicate two possible sites of action of ketamine-HCl on mitochondrial reactions; firstly low concentrations block electron transport by an interaction with the NADH-dehydrogenase enzyme complex, and secondly at higher concentrations it uncouples oxidation from phosphorylation. The overall *in vitro* effect is to prevent ATP synthesis which could in the *in vivo* situation result in the modification of impulse conduction in neurones and hence the maintenance of the anaesthetic state.

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