The Effect of Ketamine on Heart Rate in an Isolated Atrial Preparation Taken From the Rat

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Citation

A Young, A Harper. *The Effect of Ketamine on Heart Rate in an Isolated Atrial Preparation Taken From the Rat*. The Internet Journal of Cardiology. 2007 Volume 6 Number 1.

Abstract

The effect of ketamine was tested on the heart rate of an isolated atrial preparation taken from the rat. The preparation was subject to atropine and atenolol both with and without the presence of ketamine. Where atropine produced an increase in heart rate of 16.5+6.1% the addition of and atenolol produced a decrease in heart rate of 15.5+3.2% where (p=0.085, n= 3, 3), the presence of ketamine was shown to inhibit these changes. The heart rate of the preparation was also tested immediately after vagal stimulation. The right vagus nerve was stimulated at a variety of frequencies where a stimulation of 20Hz for 30 seconds produced a decrease in heart rate of 32.3+2.6%, however, when this was repeated in the presence of ketamine, there was an increase in heart rate of 11.0+0.0% where (p=0.004, n=3,3). Ketamine showed similar results when tested on ganglionic transmission of isolated cardiac parasympathetic ganglia. It is believed that ketamine may have produced an increase in heart rate by bearing an inhibitory effect on parasympathetic receptors, where the agent has a greater affinity for nicotinic-AChRs over muscarinic-AChRs.

INTRODUCTION

Neural control of the heart is subject to the regulation of sympathetic and parasympathetic divisions of the autonomic nervous system. Activation of the parasympathetic division, arising from neurons in the medulla of the brain stem, has negative chronotropic, dromotropic and ionotropic actions at the atria of the heart [1]. Conversely, activation of the sympathetic division will produce positive actions.

Intravenous (i.v.) anaesthetics are known to affect cardiac parameters under clinical conditions and in chronically instrumented animals [2, 3, 4], an increase in heart rate being the major action. However, to contradict the inclination of many who would naturally believe that an increase in heart rate was due to an increase in sympathetic activity, excitatory neurotransmission in sympathetic ganglia is blocked by i.v. anaesthetics and therefore cannot explain the increases in heart rate observed during i.v. anaesthesia [5, 6]. This effect is believed mainly to be the result of the anaesthetic inhibition of parasympathetic neurons in the peripheral nervous system that are involved in the regulation of cardiac function [7]. This concept is supported by recent reports, which reveals that the i.v. anaesthetic, ketamine, inhibits nicotinic cholinergic excitation in cardiac preganglionic parasympathetic neurons of the nucleus ambiguus of the brainstem [8]. It has also been indicated that heart rate is modulated by the parasympathetic nervous system in a nicotinic cholinergic-dependent manner, suggesting an involvement of nicotinic acetylcholine receptor (nAChR) channels [9, 10]. These findings strongly suggest an involvement of nAChRs on intracardiac neurons in the modulation of heart rate during anaesthesia, nevertheless, the effects of i.v. anaesthetics on nAChR channels in these neurons have not been studied until recently [11]. However, Weber et al, (2005) produced results from studies on actively dissociated neonatal rat intracardiac ganglion neurones grown in tissue culture. They discovered that intravenous anaesthetics inhibit nicotinic acetylcholine receptor-mediated currents and Ca^{2+} transients in rat intracardiac ganglion neurons.

It would therefore be interesting to determine if these results apply to intact ganglia, this will be explored by examining the effect of i.v. anaesthetics on intrinsic cardiac ganglions on an intact ganglion preparation at 37°C.

The hypothesis is that the ketamine will inhibit the chemical release produced by vagal stimulation throughout the parasympathetic system by blocking the nAChRs within the cardiac ganglion and mAChRs at the target tissue. However, by testing the effects of ketamine on each of these receptors, it is predicted that there will be evidence to suggest that

ketamine has a greater effect on the nAChRs at the ganglia.

METHODS

DISSECTION AND LOCATION OF GANGLIA

Female adult Wistar rats (145-187g) (Harlan U.K.) were used in this study. Rats were killed by stun and cervical dislocation, schedule 1, suitable for rodents up to 500g [12]. The heart, lungs, thymus and oesophagus (with evidence of diaphragm penetration) were removed and bathed with cold, oxygenated Krebs solution. The dissection was performed using a Wild dissecting microscope (x 40 magnification) using direct fibre optic illumination. Whilst the heart remained in an 'arrested' condition the right atrium was opened to reveal the crista terminalis and associated papillary muscles. The left atrium and ventricle tissue were removed to reveal the right atrium, associated vagus nerve and the fat pads on the surface of the atria, below which the target ganglia are located.

PROCEDURE

Solutions were delivered using a volumetric conical flask to ensure Krebs solution remained fresh. Once these procedures were carried out the Techne Circulator C-85D heating system was set to 42.5°C and the Gilson Minipulse 3 peristaltic pump was set to a flow rate of 12ml/min. These settings allowed the tissue preparation to be superfused by the desired solution at 37 +1°C in the bathing chamber which itself had a volume of 12ml. Temperature was monitored using a Fluke digital thermometer. Following the dissection the tissue was mounted in the tissue chamber for at least 30 minutes to allow for resuscitation.

SOLUTIONS

Krebs solution had a final concentration of (mM): NaCl, 118.0; $NaHCO_3$, 25.0; NaH_2PO_4, 1.13; KCl, 4.70; $CaCl_2$, 1.80; $MgCl_2$, 1.30; Glucose, 11.10 and gassed with carbogen (a gas mixture of 95% O_2/5% CO_2) which maintained a pH of 7.4

Ketamine (Vetalar) was used at concentrations 100µM and 1mM.

Atropine, atenolol, acetylcholine and epinephrine (Sigma) were used at varies concentrations specified in the results. These agents were dissolved using Milli-Q water (Millipore water purification systems, Mosheim, France) and were stored at -20°C at the following concentrations (mM): Atropine, 10.00; Atenolol, 10.00; Acetylcholine, 2.00; Epinepherine, 10.00.

VAGAL STIMULATION

The electrical stimulation was carried out by stimulating the right vagus nerve with bipolar Ag/AgCl electrodes, Grade 5. These were insulated with nitrocellulose with only the tips showing. The electrodes were attached to a DG2 via an isolated stimulator, Digitimer Ltd., set at a constant 10volts, a stimulation width of 1 msec and at a frequency of 5, 10, 20 or 50Hz. These stimulations were carried out for 10, 20 and 30 seconds for each frequency, except for 50Hz where the heart beat arrested upon application of 50Hz over a 10 second time period.

ELECTROPHYSIOLOGICAL MEASUREMENTS

The heart rate of the preparation was measured by recording the atrial action potential discharge and counting the number of action potentials on the electrocardiogram produced in a 2 second interval (see figure 4.). This was achieved by probing the heart with bipolar Ag/AgCl, electrodes, Grade 5, insulated with nitrocellulose with only the tips showing, aiming no further than 5mm from the point of the Sino atrial node which can be identified as it is found close to the crista terminalis. The electrodes were connected to a Neurolog conditioning amplifier (10Hz-5kHz) and a Tektronix 2220 (60MHz) oscilloscope. From this data the heart rate of the animal was calculated.

STATISTICS

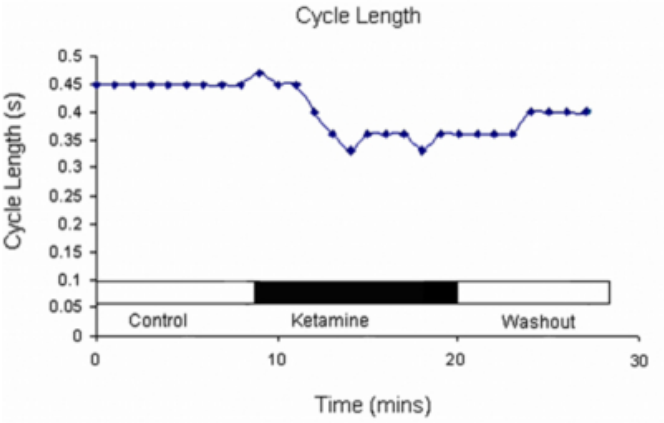
Statistics for all calculations were calculated with the students paired t-test, using Sigma Plot 5. Unless indicated, all data are expressed as MEAN+STANDARD ERROR. Data is significant if the p value is less than or equal to 0.05.

RESULTS

The affect of ketamine at concentrations of 10, 100 and 1000µM (data not shown) was examined to determine an optimum concentration for the agent, the concentration gradient identified that 100µM was the most physiologically relevant concentration to use. Fig. 1 illustrates typical results obtained from an isolated atria bathed in Krebs solution at 36.4°C.

Figure 1

Figure 1: cycle length of an isolated atrium bathed in Krebs solution at 36.4°C, illustrating the actions when 100µM ketamine is applied.



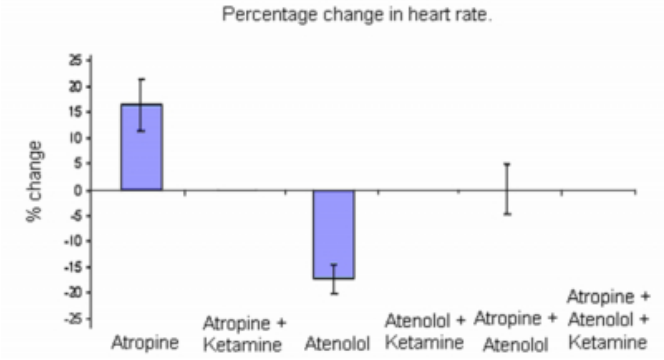
To determine the temperature sensitivity of the tissue a temperature scan from 28°C-37°C was performed. An arrhenius plot determined a linear regression analysis of r^2 =0.9619 (data not shown). The Ea value for cycle length was calculated from the linear regression plot and produced a value of 55 kJ mol^{-1} for an individual experiment.

Data was normalised and produced significant results, indicating that ketamine will increase the heart rate of an isolated preparation consistently at 100µM. Confirming the findings of previous authors [2], [3], [4], we decided to dissect a possible mechanism for this action. Interestingly, the application of ketamine together with m-AChR and -adrenoceptor antagonists produced results which are to date undocumented to the best of our knowledge (fig. 2).

The application of atropine produced an average increase in heart rate of 16.5+4.9% and atropine with ketamine produced no deviation from the control where (p=0.085, n=3, 3). The average change in heart rate upon application of atenolol was a decrease of 15.5+2.6% and atenolol with ketamine produced no deviation from the control where (p=0.028, n=3, 3). The application of atropine with atenolol produced an average change of 0.0+4.7% and the same procedure carried out with the presence of ketamine produced no deviation from the control heart rate where (p=0.089, n=3,3).

Figure 2

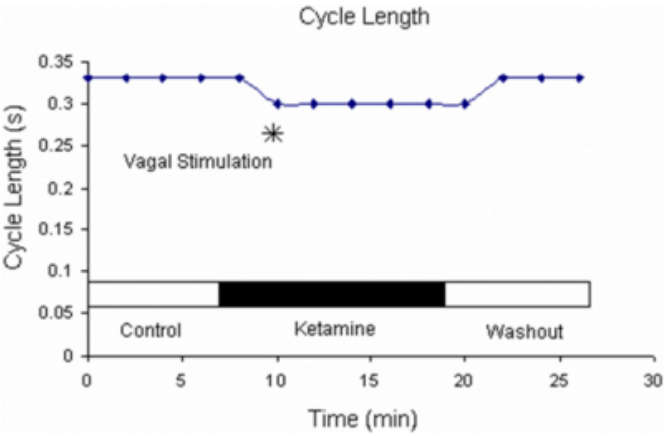
Figure 2: bar chart showing the percentage change on resting heart rate on the addition of 1µM atenolol and 1µM atropine in the presence and absence of 100µM ketamine between 34.3°C-35.6°C (n=3).



Following these results we decided to investigate the affects of ketamine on the heart rate of the preparation in the presence of vagal stimulation (fig. 3).

Figure 3

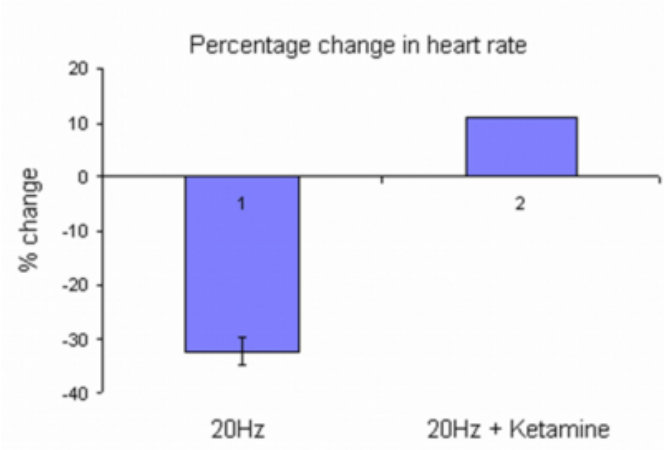
Figure 3: cycle length of atria superfused in Krebs solution at 35.2°C, illustrating the effects when a 20Hz stimulus is applied for 30 seconds in the presence of 100µM ketamine.



In agreement with Choate & Feldman (2003), we discovered that the optimum frequency of vagal stimulation was 20-30Hz. Figure 4 shows some interesting results where the heart rate of the preparation decreased by 32.3+2.6% after a 20Hz stimulation however, the same stimulation in the presence of ketamine produced an increase of 11+0.0% where (p=0.04, n=3, 3).

Figure 4

Figure 4: bar chart showing the percentage change on resting heart rate after receiving a 20Hz stimulus for 30 seconds in the presence and absence of 100µM ketamine between 33.9°C-35.2°C (n=3).



The success of these results inclined us to determine if similar effects could be detected with isolated ganglia. Isolated ganglia were examined to identify whether the number of successful action potentials was affected upon application of ketamine. The control preparation produced an average of 98+2.3% successful action potentials, on the addition of 100µM ketamine reduced this success rate to 65+1.6%, where (p=0.001, n=5,5)(fig. 5). A stimulation of 20Hz produced a success rate of 45+2.0% however, upon the addition of 100µM ketamine the rate of successful action potentials decreased to 25+1.7%, where (p=0.002, n=5,5)(fig. 6).

Figure 5

Figure 5: results showing the effect of 100µM ketamine on percentage of successful action potentials. (n=5).

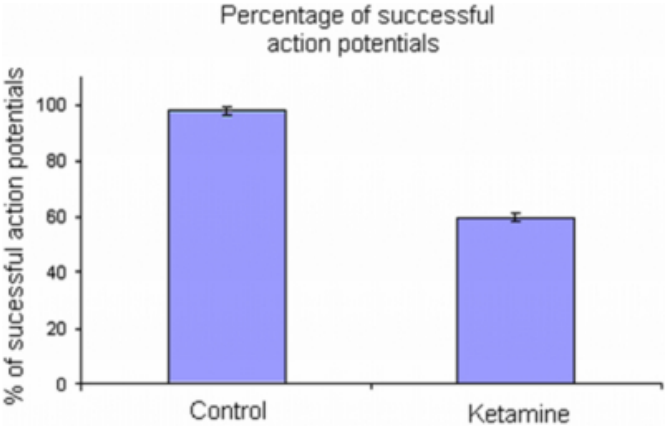
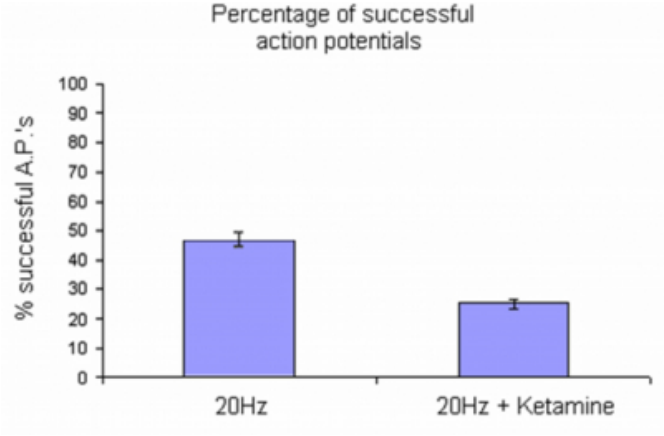


Figure 6

Figure 6: results showing the effect of 100µM ketamine on percentage of successful action potentials after a vagal stimulation of 20Hz. (n=5).



Contradictory to that of the intrinsic cardiac neurones the isolated parasympathetic cardiac ganglia produced results for a 50Hz stimulation where the mean value of successful action potentials was 28+2.54%.

DISCUSSION

APPLICATION OF ANTAGONISTS

The application of atropine produced an increased heart rate (fig. 2). This is due to the inhibition of the mAChR found on the heart tissue. By antagonising mAChRs atropine is blocking the inhibitory effect of the parasympathetic vagal nerves. However, in the presence of 100µM ketamine, the heart rate remained constant. This supports the concept that while the mAChR are being blocked by atropine the ketamine present is in fact altering the function of the -adrenoceptors, present on the heart. So far as I am aware there are no reports of i.v. anaesthetics blocking -adrenoceptors. The application of ketamine on its own has been found to increase the heart rate, however, in this instance the best way to analyse this situation would be to understand that ketamine is decreasing an already increased heart rate. One likely mechanism to enable ketamine to do this is by blocking the sympathetic -adrenoceptors on the heart. This discovery has brought forward an interesting scenario as normally the heart rate is increased by ketamine by blocking either nAChRs or mAChRs in the parasympathetic system. However, in this scenario when mAChRs are already blocked ketamine inhibits transmission in the sympathetic system leading one to believe that ketamine has more of an affinity for the parasympathetic nervous system.

The possibility that ketamine could in fact be inducing this effect by inhibiting nAChRs at the ganglion can be discounted as these were removed during dissection. Only the parasympathetic nAChRs would be present and if ketamine were to inhibit those receptors the heart rate would increase in a similar fashion to which it did when mAChRs were blocked.

The application of the -adrenoceptor antagonist, atenolol, produced a decrease in heart rate, consequently reducing the action of the noradrenaline released from the pre-synaptic terminal. This resulted in a scenario where the major neurotransmitter affecting the heart was ACh, which was acting on the mAChRs, increasing parasympathetic activity. This experience was nullified by the presence of ketamine which increased an already reduced heart rate by blocking mAChRs.

Application of both atropine and atenolol together produced two responses on the heart rate, on two instances the heart rate increased, in the other it decreased. The mean data showing no change (fig. 2). Nevertheless, upon application of ketamine the heart rate again reverted to its control level in both instances, by means discussed above.

VAGAL STIMULATION

Only frequencies above 20Hz produced the expected negative chronotropic actions. The reduction in heart rate is due to the activation of the nAChRs and subsequently the mAChRs which reduces heart rate naturally. At a stimulation of 50Hz the heart arrested, this was interesting as a stimulation of 50Hz is normally used to investigate variations in ganglionic transmission in isolated parasympathetic cardiac ganglia [14] . Nevertheless upon stimulation with 20Hz for a time period of 30 seconds the heart rate was reduced by 32.3+2.0%, this is in contrast to Choate & Feldman (2003) who found that a vagal stimulation of 5Hz produced a 29.3+0.02% decrease in heart rate, they also found that a stimulation of 10Hz often arrested the heart. However, they themselves proposed that the heart would not normally arrest unless a frequency of 30Hz was applied.

Interestingly enough, not only did the application of ketamine inhibit the decrease in heart rate produced by a 20Hz stimulation it actually increased the heart rate further. This inclines one to believe that it may be possible that under in vivo conditions the heart rate of the Wistar rat can be increased by the application of 100μM ketamine even in the presence of vagal stimulation. This is supported by Woodbury & Woodbury (1990), among others, who state that “In rats, optimal stimulus frequency is approximately 10-20 Hz”.

ISOLATED GANGLIA PREPARATIONS

The results produced from the isolated parasympathetic ganglia highlights the concept that ketamine is producing an inhibitory effect on the parasympathetic ganglia, by reducing the number of action potentials fired. In both occasions ketamine decreased the number of successful action potentials. The more physiologically relevant of the two experiments was when ketamine was applied when the preparation was being stimulated with 20Hz. Interestingly enough ketamine actually effected this preparation more.

CONCLUSION

From these preliminary results, it can be predicted that the affinity of ketamine for each receptor is as follows: nAChRs>mAChRs>-adrenoceptors. This therefore provides reasonable evidence to assume that although ketamine inhibits both the sympathetic and parasympathetic nervous systems, it in fact has a greater affinity for the parasympathetic system, the reason why i.v. anaesthetics such as ketamine increase heart rate. As far as we are aware this has not been documented to date.

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