INFLUENCE OF ANAESTHETICS ON THE INCIDENCE OF REPERFUSION-INDUCED ARRHYTHMIAS AND SUDDEN DEATH IN RATS

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Summary: We compared the influence of pentobarbital (P, 60 mg kg^{-1}), urethane (U, 1.8 g kg^{-1}) and a contribution of diazepam with ketamine hydrochloride (D+K, 10+100 mg kg^{-1}) anaesthesia on the incidence of ischaemia/reperfusion induced arrhythmias in rats. In anaesthetized rats myocardial ischaemia was produced by a 6 min ligation of the left main coronary artery, followed by 5 min of reperfusion. The incidence of reperfusion induced ventricular fibrillation (VF) and ventricular tachycardia (VT) was markedly decreased in the D+K anaesthetized animals compared to the P anaesthetized group (VF: 46% vs 89%, VT: 64% vs 94%). The mean blood pressure (MBP) before coronary ligation was significantly lower in U anaesthetized animals (72 ± 3.5 vs 102 ± 4.1 and 108 ± 5.9 mmHg in P and D+K, respectively), the MBP recovery was the best in the D+K anaesthetized group. In summary, the U anaesthesia markedly decreased the MBP of the animals compared to P or D+K, while the incidence of ischaemia/reperfusion induced life threatening arrhythmias was the highest in the P anaesthetized rats.

Key Words: Reperfusion arrhythmias - Rats - Pentobarbitone - Diazepam - Ketamine - Urethane

INTRODUCTION

Restoration of the coronary blood flow following a brief ischaemic period results in severe, life threatening arrhythmias in experimental animals (1). These arrhythmias can be consistently elicited in anaesthetized rats and may represent arrhythmias developing after surgical reperfusion, thrombolysis or spontaneous release of coronary vasospasm in humans. Most often they appear in the form of ventricular fibrillation, ventricular tachycardia and premature ventricular beats. Reperfusion arrhythmias in anaesthetized rats are easily reproducible thus giving a good opportunity for examining the effects of different agents on these types of cardiac arrhythmias.

In rats the most severe reperfusion arrhythmias appear following 5-6 min of preceding ischaemia. The mechanism leading to the development of these arrhythmias is still the subject of speculation, both reentry and increased automaticity can be responsible for the process (2). It is well established that the occlusion of the coronary artery creates a significant electrical inhomogeneity between the ischaemic and non-ischaemic myocardium that seems to be rapidly and further exacerbated by reperfusion. This heterogeneity can be the electrophysiologic basis supporting the theory that reperfusion arrhythmias develop via reentry mechanisms. Other studies claimed that increased automaticity plays an important role in the formation of reperfusion arrhythmias (3,4). This contradiction seems to be resolved by a study from Kaplinsky *et al*

(5) who, on the basis of their experiments on greyhound dogs suggested that reperfusion arrhythmias have two types. The first type is reentry arrhythmias appearing within the first minute following the release of occlusion with increased incidence of ventricular fibrillation, the second type is arrhythmias caused by enhanced automaticity, occurring between the second and seventh minute after the onset of reperfusion having lower incidence of venticular fibrillation.

An earlier study in our laboratory (6) showed that there is considerable difference between conscious and pentobarbitone anaesthetized animals in the survival rate and in the number of animals which developed irreversible ventricular fibrillation in response to acute coronary ligation. Thus the origin of some discrepancies in the literature concerning the results of reperfusion arrhythmia studies may also be found in the different anaesthetization methods applied in those studies.

Little is known about the influence of different anaesthetics on reperfusion induced arrhythmias. Thus the aim of the present experiments was to compare the influence of three anaesthetic methods, commonly used in cardiovascular studies, i.e. diazepam + ketamine hydrochloride, pentobarbitone, as well as urethane anaesthesia on reperfusion induced arrhythmias in rats.

MATERIALS AND METHODS

Animals

The present study was performed on male Sprague-Dawley CFY rats, weighing 300-350 g. The animals were housed six to a cage and allowed to have tap water and laboratory rat chow (Altromin, Gödöllő, Hungary) *ad libitum* until the experiment. The animals were handled according to a protocol reviewed and approved by the Ethical Committee for the Protection of Animals in Research of the Albert Szent-Györgyi Medical University, Szeged, Hungary.

Anaesthetic agents

Pentobarbitone sodium (P, CEVA, France, 60 mg kg^{-1} in a volume of 2 ml kg^{-1}) was administered intraperitoneally. Diazepam + Ketamine hydrochloride (D+K, RICHTER , Hungary, 10 mg kg^{-1} and 100 mg kg^{-1} intraperitoneally, respectively, in a volume of 4 ml kg^{-1}). Urethane (U, REANAL, Hungary) was administered also intraperitoneally (1.8 g kg^{-1} in a volume of 4 ml kg^{-1}).

Coronary artery ligation and reperfusion:

Acute coronary ligation and reperfusion was produced according to Kane *et al* (7). After tracheal cannulation thoracotomy was performed in the fourth intercostal space and the heart was exposed. A loose loop of 5-0 atraumatic silk (K 890 H, Ethicon, England) was placed around the left main coronary artery, approximately 2 mm from its origin. Both ends of the ligature were led out of the thoracic cavity through a flexible tube. The heart was set back in its place

and artificial respiration started immediately with 60 strokes min^{-1} (Harvard rodent ventilator, model 683, Harvard Apparatus, Southnatick, MA, USA).

Blood pressure was measured in the carotid artery using a pressure transducer (Gould-Statham, P23ID, Hugo Sachs Electronik, March-Hugstetten, Germany) and was recorded on an oscillographic recorder (Watanabe, WTR 331, Hugo Sachs Electronik). The catheter was filled with isotonic saline containing heparin (500 IU ml^{-1}), but the animal was not heparinized. The standard electrocardiogram (lead II) was also recorded with the help of subcutaneous needle electrodes.

After stabilization of the blood pressure and heart rate that took approximately 10 min, the loose loop was tightened and fixed by clamping on the silk and thus local myocardial ischaemia was produced. After 6 min of myocardial ischaemia the ligature was released and 5 min of reperfusion followed.

The incidence of arrhythmias was registered both during occlusion and reperfusion in accordance with the Lambeth conventions as venticular tachycardia (VT), ventricular fibrillation (VF) and other types of arrhythmias including single extrasystoles, bigeminy, salvos and bradycardia (8). The onset and duration of arrhythmias were also measured (9). An arrhythmia score was used to evaluate the incidence and duration of arrhythmias by giving a grade to each animal as follows: 0 = no arrhythmias; 1 = < 10 sec VT or other types of

arrhythmias, no VF; 2 = 11-30 sec VT or other types of arrhythmias, no VF; 3 = 31-90 sec VT or other types of arrhythmias, no VF; 4 = 91-180 sec VT or other types of arrhythmias, and/or < 10 sec reversible VF; 5 = > 180 sec VT or other types of arrhythmias, and/or > 10 sec reversible VF; 6 = irreversible VF.

No attempt was made artificially to revert ventricular fibrillation during ischaemia or reperfusion. At the end of the experiments the hearts were excised and after tightening the ligation they were perfused retrogradely with 10 ml isotonic saline and 2 ml of 96% ethanol through the aorta for determining the extent of the perfusable and non-perfusable areas (9). According to our previous investigations, using ethanol instead of formaldehyde for the retrograde perfusion does not change the perfusable areas significantly. The bright white area represented the perfusable area of the heart while the colour of the non-perfusable myocardium did not change. The hearts were cut along the epicardial border line and the wet weight of the non-perfusable myocardium was expressed as the percentage of the total weight of the ventricles. The weight of the non-perfusable myocardium was not significantly different among the three groups and varied between 37.7 ± 2.13 and 38.6 ± 1.77 %. If the perfusion proved that the ligature was at an inadequate place (the whole heart could be perfused) and no change in the ECG (T-wave elevation, QRS distorsion) and no decrease in blood pressure occurred upon tightening of the ligature, the animal

was excluded from the evaluation of the experiments. On the basis of these criteria altogether 4 animals were excluded.

Statistical evaluation

The incidence of arrhythmias was expressed in percent and compared by using the χ^2 method. All other parameters were expressed as mean ± standard error of the mean (SE) and after analysis of variance compared by means of the modified "*t*" statistic of Wallenstein *et al* (10).

RESULTS

Haemodynamic parameters

Urethane anaesthesia resulted in a significantly lower mean blood pressure (MBP) already at the end of the stabilization period that remained for the occlusion period as well (TABLE 1). During reperfusion this significant difference in MBP was diminished. The decrease in blood pressure in D+K anaesthetized animals was significantly less expressed during coronary artery occlusion and during reperfusion compared to pentobarbitone anaesthetized rats (TABLE 1). There was a modest elevation of the basal heart rate in the pentobarbitone anaesthetized group, but there was no significant difference in the heart rate response among the three groups during ischaemia or reperfusion (TABLE 1).

The pressure rate index (PRI) is the product of mean blood pressure and heart rate (mmHg x min^{-1} x 1000^{-1}) and corresponds to the oxygen and energy demand of the myocardium. The PRI was significantly lower in the urethane anaesthetized rats before occlusion compared to pentobarbitone anaesthetized animals and during ischaemia it was significantly higher in D+K anaesthetized rats than in the pentobarbitone anaesthetized group (TABLE 1).

Arrhythmias during occlusion

The arrhythmias during coronary artery ligation occurred in the sixth minute in all groups. During the 6 min occlusion period 5 animals died in the

pentobarbitone anaesthetized group while in the other two groups all animals survived occlusion (TABLE 2). The incidence of ventricular tachycardia and ventricular fibrillation was significantly less in both the D+K and urethane anaesthetized group compared to the pentobarbitone anaesthetized animals (TABLE 2). The incidence of other types of arrhythmias, including sporadic extrasystoles, bigeminia and salvos, was significantly higher in the pentobarbitone anaesthetized animals than in the urethane anaesthetized group. The arrhythmia scores of the pentobarbitone anaesthetized animals were markedly higher during occlusion than of the animals in the other two groups (TABLE 2).

In the D+K and urethane anaesthetized group the arrhythmias during myocardial ischaemia appeared later than in the pentobarbitone group (TABLE 3). The duration of the period characterized by different arrhythmias during ischaemia was shorter both in the D+K and urethane anaesthetized group than in the pentobarbitone anaesthetized group (TABLE 3).

Arrhythmias during reperfusion

Reperfusion induced arrhythmias appeared within 10-30 seconds following the release of the ligature and were more severe than those developing during the 6 minutes of ischaemia. In the D+K anaesthetized group the incidence of ventricular fibrillation and tachycardia during rerperfusion were significantly lower than pentobarbitone or urethane anaesthetized animals

(TABLE 2). As a result, the survival rate during reperfusion was significantly better in the D+K anaesthetized group and the arrhythmia score was also significantly smaller than in the other two groups (TABLE 2). There was no significant difference in the duration of reperfusion arrhythmias in the surviving animals in the three groups. However, ventricular fibrillation did not develop in the D+K and urethane anaesthetized surviving animals in contrast to the pentobarbitone anaesthetized animals (TABLE 3).

DISCUSSION

The experimental induction of reperfusion arrhythmias is a well accepted and useful tool for measuring the efficacy of potentially antiarrhythmic drugs, since these arrhythmias can be reproduced under precise conditions in both in vitro and in vivo experiments. The severity of reperfusion arrhythmias depends on the time course of the preceding coronary occlusion (2). Our intention was to produce serious and reproducible arrhythmias during reperfusion. Six minutes of preceding coronary occlusion was applied in the present study that provided severe and rapidly appearing arrhythmias after the release of the coronary ligation while the ischaemic period was not long enough for a significant amount of severe ischaemic arrhythmias to develop (11).

The majority of the in vivo experiments investigating arrhythmias during occlusion and reperfusion are performed on anaesthetized animals, thus it seems to be important to consider not only the analgesic and anaesthetic effects of the anaesthetics but also their general depressant action on the autonomic nervous system.

In cardiovascular experimental work pentobarbitone, urethane, diazepam and ketamine are widely used anaesthetic agents. Few data can be found in the literature concerning the effects of these anaesthetic agents on reperfusion induced arrhythmias. In our experiments we compared the effects of three

anaesthetic methods, i.e. urethane, diazepam and ketamine combination, and pentobarbitone on ischaemia and reperfusion-induced arrhythmias in rats.

Sodium pentobarbitone has well known autonomic reflex decreasing and vagolytic effects, of which the most prominent manifestation is its vagolytic action on the heart, i.e. the elevation of heart rate. In our experiments we also found a moderate elevation in the basal heart rate of sodium pentobarbitone anaesthetized animals. It is also a membrane depressant on myocytes and reduces the responsiveness of these cells to sympathetic stimulation, thus having some kind of antiarrhythmic effect (12). Indeed, Siegmund *et al* (6) demonstrated that during pentobarbitone anaesthesia, compared to conscious animals, a significantly lower number of animals developed irreversible ventricular fibrillation during the first twenty minutes of myocardial infarction. Dawson *et al* (13) have shown that pentobarbitone affects the ventricular fibrillation threshold in dogs thus influencing the vulnerability of the myocardium to ventricular fibrillation.

In our experiments comparing three different types of anaesthesia, however, the incidence of ventricular fibrillation and tachycardia was the highest in the pentobarbitone anaesthetized animals both during occlusion and reperfusion. Hunt and Ross (14) found that pentobarbitone prolonged the QT interval without changing ventricular refractoriness in dogs, while Saarnivaara and Lingren (15) found that thiopental, another barbiturate, also prolongs the

QT interval during clinical induction of anaesthesia. This mechanism possibly predisposes the myocardium to the development of serious ventricular dysrhythmias.

Diazepam is commonly administered for sedation to patients having acute cardiac ischaemia. Some studies have reported that the beneficial effects of diazepam lie not only in its anxiolytic action but it also has direct cardiac effects by reducing contractility and increasing myocardial blood flow in *in vivo* and in isolated heart investigations as well (16, 17), thereby improving the prospects of the ischaemic myocardium to survive. The identification of peripheral benzodiazepine receptors in the heart seems to support this theory (18). Diazepam have been found to exert antiischaemic effects in patients with documented coronary artery disease (19). Diazepam in combination with ketamine is widely used for anaesthetic purposes in human interventions as well. Diazepam have been found to block the positive inotropic and chronotropic effects of ketamine that has excellent analgesic and amnesic effects thereby suggested as an alternative anaesthesia in open-heart surgery (20). Our findings, i.e. the haemodynamic parameters of diazepam + ketamine anaesthetized animals were the most impressive during ischaemia and at the end of the reperfusion period, correspond with the beneficial haemodynamic effects of diazepam on the ischaemic myocardium outlined in the literature. Moreover, the combination of diazepam and ketamine induced remarkably fewer and less

severe arrhythmias in the present study. The incidence of ventricular fibrillation and ventricular tachycardia was significantly lower in D+K anaesthetized rats than in the pentobarbitone anaesthetized animals resulting in significantly increased survival rate.

Urethane is reported to have marked depressive effect on the cardiovascular system (21, 22, 23). Indeed, we found that the blood pressure and the pressure rate index was significantly lower in the urethane anaesthetized animals than in the pentobarbitone or in the diazepam + ketamine anaesthetized rats. This effect of urethane on the cardiovascular system may have great importance not only for investigators measuring haemodynamic parameters but also for those who study arrhythmias in anaesthetized animals. Goes *et al* (24) found that the duration of reperfusion arrhythmias following 10 min occlusion significantly increased in isolated rat hearts taken from urethane anaesthetized animals compared to hearts taken from pentobarbitone anaesthetized animals. In our experiments, the duration of these arrhythmias in urethane anaesthetized rats did not differ significantly from that in pentobarbitone anaesthetized animals. However, the length of ventricular fibrillation and the total length of arrhythmic attacks during reperfusion in the surviving pentobarbitone anaesthetized animals was significantly longer compared to the urethane anaesthetized animals. In our investigations there was no significant difference in the incidence of reperfusion arrhythmias between the pentobarbitone and

urethane anaesthetized groups that corresponds to the findings of the in vitro study by Goes *et al* (24).

In conclusion, since the combination of diazepam and ketamine has a remarkable activity in decreasing the incidence of ischaemia and reperfusion induced arrhythmias, this type of anaesthesia may be recommended mainly for surgical interventions in experiments where avoiding ischaemia and reperfusion induced arrhythmias is desirable, i.e. haemodynamic investigations during myocardial infarction in animal experiments. Pentobarbitone anaesthesia has advantages in cardiovascular experimental work for studying arrhythmias and potentially antiarrhythmic drugs during occlusion and reperfusion, since it allows the development of severe ventricular arrhythmias providing the opportunity for the investigated drugs to diminish dysrhythmias themselves. The haemodynamic parameters remain sufficiently stabile throughout pentobarbitone anaesthesia, while one must be aware of the cardiovascular depressive effects of anaesthesia when applying urethane for experimental purposes.

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