EFFECT OF HALOTHANE ON MYOCARDIAL INFARCT SIZE IN RATS

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ABSTRACT

The effect of halothane on myocardial infarction caused by ligation of the left descending coronary artery was studied in rats. The extent of infarction was quantified 48 hours after ligation of the artery by planimetric measurement of left ventricular slices stained with nitrobluetetrazolium. Animals exposed to halothane one per cent for three hours after the coronary ligation were compared with a control group which received halothane for only 5–7 minutes during surgery. It was found that halothane caused a small increase in infarction size (31.3 ± 1.5 per cent of the left ventricle compared to 25.7 ± 2.3 per cent, $p < 0.05$). This effect was accompanied by a decrease in systolic blood pressure (91 ± 2 mmHg compared to 113 ± 3 mmHg, $p < 0.001$). Heart rate did not change significantly.

Analysis of our results in comparison to previously reported data on the effect of halothane on myocardial ischaemia in different experimental conditions shows that halothane may produce beneficial as well as detrimental effects on ischaemic injury to the myocardium. The latter can result when the drug causes marked hypotension in the absence of a significant decrease in heart rate.

KEY WORDS: ANAESTHESIA, halothane; HEART, myocardial infarct, halothane.

BLAND AND LOWENSTEIN¹ demonstrated in dogs that halothane decreases the severity of myocardial ischaemia produced by repeated coronary occlusions and estimated by epicardial ST-segment mapping. The importance of the problem of choice of anaesthetic for patients with ischaemic heart disease makes it interesting to assess the effect of halothane on ischaemic injury to the myocardium using a direct morphological method. In this study, the histochemical method by Nachlas and Schnitka² was used.

METHODS

Experiments were conducted on Sprague-Dawley male rats, weighing 300–350 g. Myocardial infarction was produced by a method similar to that described by Johns and Olson³ and Selye with co-workers.⁴ Each animal was initially anaesthetized with four per cent halothane which was delivered into a plastic bag enclosing the cage. After a two minute exposure, the animal was fastened to an operating board and anaesthesia was continued with two per cent halothane for approximately five minutes during coronary ar-

tery ligation. Halothane was delivered through a tightly fitting face-mask while respiration was maintained with a respirator pump (Harvard Apparatus, Model 607). As a vaporizer, a recently calibrated Fluotec (Cyprane, Inc. FR type) was used. A reservoir bag was interposed between the pump and the vaporizer. A cranio-caudal incision to the left and parallel to the sternum was made through the skin and pectoral muscles and a purse string suture was placed around the incision site (Figure 1). A left thora-

FIGURE 1 Incisions necessary for exteriorizing the heart. (1) Fourth rib; (2) Fifth rib; (3) Sternum; (4) Purse string suture. Thick interrupted lines – incisions for thoracotomy.

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cotomy was done through the fourth intercostal space. The fourth and fifth ribs were separated and their cartilages were transected approximately 0.5 cm to the left of the sternum. After exteriorization of the heart, the left descending coronary artery was ligated 5–6 mm from its origin with 6-0 Ethicon cardiovascular silk suture. The heart was replaced in the thorax, and the previously placed suture was closed. The period when the thorax remains open is short enough that the authors of this method^{3,4} did not use artificial ventilation. The rats were sacrificed 48 hrs after ligation of the coronary artery, when the necrotic process was well established.⁵

Myocardial infarct size was estimated on tissues stained with nitrobluetetrazolium (NBT) reaction for the presence of intracellular dehydrogenase activity according to Nachlas and Schnitka.² At the time of sacrifice, each rat was injected with heparin (100 units). Five minutes later the heart was excised, washed and placed in saline at 37°C for twenty minutes to promote development of rigor mortis to provide a uniform state of contraction among hearts. After rigor mortis developed, the heart was blotted dry and placed in the freezer for 20 minutes. When firm, the heart was cut from apex to the occluding ligature into three transverse slices. The slices were incubated for 15 minutes at 37°C in a potassium phosphate buffered solution of 0.05% NBT. Incubation with NBT resulted in an intense staining reaction in undamaged regions of the heart while areas of necrotic tissue appeared as clearly delineated pale zones. After fixation in 10 per cent buffered formalin, color photographs of the upper surface of each slice were taken with a polaroid camera (Polaroid Corp., MP-4) at a magnification of 10× (Figure 2). Total and infarcted area of the left ventricle were measured. The area of damaged myocardium on all three slices was expressed as a percentage of the total area of the left ventricular sections. The measurements were made by planimetry using a sonic digitizer (Graf-pen) interfaced to a Hewlett Packard 9825A calculator system.

In a special series of experiments, when planimetric measurements of infarct size had been done, the unstained portions of the left ventricular myocardium were sharply dissected from the slices with the use of a micromanipulator and then weighed. The infarct volume and its percentage of the left ventricle were then calculated and compared with the results obtained from the planimetric method (Table I). Correlation be-

FIGURE 2 Entire heart slice (10×) with and infarction (48 hrs) demonstrated by nitrobluetetrazolium staining.

TABLE I
COMPARISON OF TWO METHODS OF INFARCT SIZE MEASUREMENTS

No. of exp.	Infarct size, % of LV	
	Sonic planimetry (Graf-pen)	Dissection with micromanipulator and weighing
1	21	22
2	25	23
3	26	24
4	32	25
5	27	25
6	12	7
7	10	7
8	24	29
M ± S.E.	20.9 ± 5.1	21.5 ± 2.8

$$r = 0.91; p < 0.01.$$

tween the two sets of results was sufficiently high ($r = 0.91$, $n = 8$, $p < 0.01$).

The systolic blood pressure and heart rate were measured by an indirect tail-cuff method, using a programmed electro-sphygmomanometer (Narco Bio-Systems, Inc. PE-300). A tachograph (Grass, 7P4F) was used to follow changes in heart

rate. Both parameters were recorded with a polygraph (Grass, model 7D). Before the measurement, the rat tail was heated for five minutes by a 75-watt light bulb placed 10–12 inches away from it.

The animals were randomly divided into two groups. The control group received halothane two per cent for about five minutes during the surgical procedure only; the halothane group received halothane one per cent for an additional three hours after ligation. The individual responsible for measuring the size of the infarction was "blinded" to the treatment. For the exposure to halothane, rats were placed in a transparent plastic cage connected to a calibrated Fluotec® vaporizer with air passing through the vaporizer and the cage at a rate of two l/min. Rats of all groups were heated with an electric blanket placed under the cages. Rat temperature was monitored with rectal thermistor probes connected through a switch box to a telethermometer (Yellow Springs Inst. Co., Model 43). The temperature was maintained at 37°C.

Halothane was furnished by Ayerst Laboratories, Inc. Nitrobluetetrazolium was purchased from Sigma Chemical Corp.

RESULTS

Thirty-nine rats were used, of which thirty survived coronary occlusion. Mortality rate in both groups of animals was almost equal. In the halothane group 15 rats survived and 5 died compared with 15 and 4 respectively in the control group.

In the control group infarct size was 25.7 ± 2.3 per cent of the left ventricle (Figure 3). Halothane caused a small increase in infarction size (31.3 ± 1.5 per cent vs 25.7 ± 2.3 per cent, $p < 0.05$). Arterial blood pressure measured at the end of the three hour exposure to halothane was 91 ± 2 mm Hg compared with 113 ± 3 mm Hg in the control ($p < 0.005$). The difference between heart rate in the halothane (388 ± 9 beats/min) and control (402 ± 5 beats/min) groups was not statistically significant.

DISCUSSION

Our study has demonstrated that myocardial infarct size in rats anaesthetized with one per cent halothane for three hours was greater than in control animals.

Table II compares data from most of the studies examining halothane in the setting of

FIGURE 3 Effect of halothane one per cent for three hours on myocardial infarct size. Values are means $\pm$ SE.

experimental ischaemia. It is seen that the beneficial effect of halothane on myocardial ischaemia, first reported by Bland and Lowenstein,¹ is a matter of controversy. Some of the authors⁷⁻⁹ could not confirm any improvement in the ischaemic region; in fact, they found changes that may be regarded as a tendency toward deterioration of energy demand/supply balance.

It is commonly held at present that factors increasing the oxygen demand increase the infarct size. Conversely, factors improving the oxygen supply reduce the infarct size. According to Opie,¹¹ "confusion arises from the delicate balance between salutary and harmful effects of the specific interventions". Halothane appears to be an illustration of this. It may cause pronounced changes in heart rate and arterial pressure that have the opposite effects on ischaemia. It is well known that a decrease in heart rate has a beneficial effect on ischaemia and a decrease in arterial pressure a detrimental effect, due to reduction of the collateral blood supply.¹²⁻¹⁴ In our study, halothane did not change heart rate significantly and decreased arterial pressure. Therefore, it is conceivable to assume that the increase of myocardial infarct size was related to the reduced arterial pressure.

In a separate group of seven rats, we measured

TABLE II
HALOTHANE (0.75-1 percent) TREATMENT IN EXPERIMENTAL MYOCARDIAL HYPOXIA

Authors	Model	Index of Ischemia	Index Change	Heart Rate (Beats/min)		Arterial Pressure (mm Hg)	
				Control	Halothane	Control	Halothane
Bland, Lowenstein ¹	Coronary occlusion in dogs	S T-segment	Improved	160	139	119	108
Reves <i>et al.</i> ⁷	Coronary occlusion in dogs	S T-segment Pm _{O₂}	No change Decreased (trend)	130	129	106	106
Moores, <i>et al.</i> ⁸	Hypoxemia in swine	Pm _{CO₂}	Increased	Paced		Controlled	
Tinker, Harrison ⁹	Coronary occlusion in dogs	ATP	Decreased (trend)	157	153	116	69
Smith, <i>et al.</i> ¹⁰	Coronary occlusion in dogs	Coronary venous Po ₂	Increased (trend)	158	135	124	79

Pa_{O₂} and Pa_{CO₂} in the samples of blood taken from the carotid artery after three hours of exposure to one per cent halothane. Pa_{O₂} was 12.24 ± 0.8 kPa (92 ± 6 mm Hg) and Pa_{CO₂} 6.25 ± 0.4 kPa (47 ± 3 mm Hg). These data agree well with results reported by White and associates⁶ and show that there was no significant hypoxia. Therefore, the probability that the hypoxia might be the cause of increase in infarct size is extremely remote.

It is of interest that the authors⁷⁻⁹ (see Table II) who reported the tendency for increased severity of myocardial ischaemia after halothane did not observe significant changes in heart rate. At the same time, in experiments of Bland and Lowenstein¹ and Smith and associates¹⁰ who reported a beneficial influence of halothane on regional myocardial ischaemia, there was a pronounced heart slowing effect. It is possible to suggest that the disparity between the results obtained in our experiments and those of Bland and Lowenstein is also due to the difference in heart rate changes under the influence of halothane.

Very pronounced heart slowing effect of halothane observed in some open-chest experiments on dogs^{1,10} is probably due to excessively high baseline heart rate—about 160 beats/min (normal canine heart rate is 70-100 beats/min). In unanesthetized dogs with normal baseline heart rate, halothane may even cause tachycardia.^{15,16}

It would appear that when heart rate is decreased after halothane, the drug has a beneficial effect on ischaemic myocardium and when heart rate does not change, there is no benefit. That is in accordance with many experimental and clinical

observations concerning the role of heart rate in myocardial ischaemia.¹⁷ Loeb and associates¹⁸ have recently shown that for similar increases in myocardial oxygen consumption, the stress from increased heart rate results in more myocardial ischaemia than the stress induced by increase in the afterload.

The possible detrimental effect of halothane on myocardial ischaemia is associated with its hypotensive effect, leading to decrease in coronary perfusion pressure. It has been shown recently that halothane depresses contractile function in the region of myocardial ischaemia caused by reduction in coronary inflow. This effect was prevented when the low level of perfusion to the ischaemic zone was controlled with a pump so that halothane could not cause any additional reduction in coronary inflow¹⁹. The authors write, "We believe that if halothane produces severe depression in situations wherein there is ischaemic myocardium, it is due to a combination of peripheral effects and generalized myocardial depression. We conclude that halothane does not produce greater functional depression in regions of myocardial ischaemia by a direct effect." These data are supported by clinical experience. Prys-Roberts and associates²⁰ in a study with hypertensive patients, have shown that halothane induced hypotension may be a critical factor in reducing coronary blood flow to a degree where electrocardiographic evidence of ischaemia is present.

Analysis of our results in comparison with the data obtained by other authors regarding the

effect of halothane on myocardial ischaemia in different experimental conditions shows that halothane may produce beneficial as well as detrimental effects on injury to the myocardium by ischaemia. The latter can result when the drug causes marked hypotension in the absence of significant decrease in heart rate.

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RÉSUMÉ

L'influence de l'administration d'halothane sur l'importance de l'infarctus produit par la ligature de la descendante antérieure chez le rat a été évaluée par des mesures planimétriques sur des tranches ventriculaires colorées au bleu de nitrozoium, 48 heures après la ligature de la coronaire. Aux fins de l'étude, on a comparé un groupe de rats auxquels l'on a administré de l'halothane à un pour cent durant 3 heures après la ligature de la descendante antérieure à un second groupe qui ont reçu l'halothane seulement durant la période de 5 à 7 minutes pendant laquelle on procédait à la chirurgie. Nous avons observé des infarctus plus importants chez les animaux ayant reçu l'administration prolongée d'halothane que chez ceux ne l'ayant reçu que pendant la chirurgie ($31.3 \pm 1.5\%$ du ventricule gauche vs $25 \pm 2.3\%$; $p < 0.5$). Nous avons également observé une diminution de la pression artérielle systolique chez ceux ayant reçu l'halothane durant 3 heures (91 ± 2 mm Hg vs 113 ± 3 mm Hg; $p < 0.001$). La fréquence cardiaque était comparable dans les deux groupes.

L'interprétation de ces résultats par rapport aux publications antérieures au sujet des effets de l'administration d'halothane sur l'ischémie myocardique, dans un contexte expérimental différent, indique que l'halothane peut avoir des effets bénéfiques ou nocifs sur l'étendue du dommage myocardique. Les effets nocifs surviennent lorsque cet agent produit une hypotension marquée non accompagnée d'un ralentissement parallèle de la fréquence cardiaque.