DELTA OPIOID EXTENDS HYPOTHERMIC PRESERVATION TIME OF THE LUNG

To test the hypothesis that a delta opioid, DADLE ([D-Ala², D-Leu⁵]-enkephalin), could protect tissue from ischemic damage during hypothermic lung preservation, we studied three groups of rats. In group 1 ($n = 8$), lung function was studied immediately after harvesting. In group 2 ($n = 8$), the lung was flushed with 4° C Euro-Collins solution and preserved for 24 hours. In group 3 ($n = 8$), the lung was flushed with 4° C Euro-Collins solution plus DADLE (1 mg/kg) and preserved for 24 hours. Lung function was studied by using a living rat perfusion model. Venous blood from the host rat perfused the pulmonary artery of the isolated lung. Blood from the isolated lung was returned to the carotid artery of the host rat with a roller pump. Severe pulmonary edema, hemorrhage, and occlusive pulmonary artery resistance occurred in group 2 within 30 minutes of perfusion. Perfusion studies were carried out for more than 60 minutes in groups 1 and 3. Pulmonary blood flow was lower in group 2 than in either group 1 or group 3. Pulmonary vascular resistance was much higher in group 2 than in groups 1 and 3 ($p < 0.05$). Airway pressure and airway resistance were much higher in group 2 than in groups 1 and 3 ($p < 0.05$). Airway resistance was also higher in group 3 than in group 1 after 20 minutes of perfusion ($p < 0.05$). Oxygen tensions from the pulmonary vein of the isolated lung in group 2 were lower than those in groups 1 and 3 ($p < 0.05$). Alveolar-arterial oxygen difference was much higher in group 2 than in groups 1 and 3 ($p < 0.05$). Lung tissue wet/dry weight ratio after perfusion was much higher in group 2 than in groups 1 and 3. The results clearly show, for the first time, that DADLE can effectively enhance hypothermic lung preservation in rats. (*J THORAC CARDIOVASC SURG* 1996;111:259-67)

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During the past 30 years, various methods have been developed for preserving solid organs for transplantation. In many cases, these methods have included flushing or perfusing organs with cold crystalloid solution or plasma. The recent development of the University of Wisconsin solution now permits kidneys to be stored for 72 hours and livers

for 24 hours in animal studies. However, safe clinical preservation times for the heart and lungs are still limited to 4 to 6 hours, no matter what solution is used (including University of Wisconsin solution, Euro-Collins solution, low-potassium dextran solution, University of California-Los Angeles solution, and the above solutions with many additives). In our normothermic multiple organ preservation studies, we found that a delta opioid, DADLE ([D-Ala², D-Leu⁵]-enkephalin), could extend tissue survival time substantially. The principal mechanism by which DADLE extended tissue survival time was thought to be related to its ability to protect tissue from ischemic damage in several aspects.¹ Because of technical difficulties, normothermic multiple organ preparations are used by only a few groups around the world,^{2,3} and the use of DADLE in such preparations is limited at the present time. To test the hypothesis that DADLE could also protect

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tissue from ischemic damage during hypothermia, we performed an isolated lung preservation study and found that DADLE could substantially enhance hypothermic lung preservation. This article reports the results of that study.

Methods

Adult Sprague-Dawley rats (250 to 300 gm) were allowed free access to food and water before operation. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Preparation of hypothermic isolated lung storage. The rat was anesthetized by intraperitoneal injection of sodium pentobarbital (50 to 55 mg/kg). The cervical trachea was cannulated, and the animal's lungs were ventilated with room air by means of a rodent respirator. Tidal volume of 2.5 to 3.5 ml, rate 40 to 50 rpm, and peak end-expiratory pressure of 0.5 cm H₂O were maintained. The retrosternal space was exposed by blunt dissection, and two large straight clamps were applied in a parallel fashion across the sternum. Care was taken not to injure the lungs and vessels beneath. The chest was opened through a median sternotomy. After intravenous administration of 1 mg heparin sodium, the inferior pulmonary ligaments were carefully divided. The left superior vena cava was divided and ligated. The hilum of the left lung was approached anteriorly, and the vessels and bronchus were separated by blunt dissection. The left pulmonary artery was dissected, and a suture was placed around it. The main pulmonary artery was transected through the transverse sinus, and a cannula was placed in the left pulmonary artery through the transected main pulmonary artery. The suture around the left pulmonary artery was tied, and the left atrium was partly excised for decompressing. The left lung was flushed with 4° C preservation solution until the lung was completely white. The lung was removed and preserved at 4° C for 24 hours before functional studies.

Technique of isolated lung function studies. A perfusion apparatus consisting of a living host rat and a temperature-controlled, humidified chamber was used for isolated lung function studies. The host rat was anesthetized by intraperitoneal injection of sodium pentobarbital (50 to 55 mg/kg). The cervical trachea was cannulated, and the animal's lungs were ventilated with room air by a rodent respirator. Tidal volume of 2.5 to 3.5 ml and rate of 40 to 50 rpm were maintained. The right internal jugular vein was cannulated with a 2 mm catheter. Heparin sodium 3 mg/kg was infused intravenously, and both carotid arteries were cannulated with 1 mm catheters. The catheter in the right carotid artery was connected to a pressure transducer for arterial blood pressure monitoring. The host rat was placed above the isolated lung in such a way that blood from the host rat was withdrawn by a constant hydrostatic pressure of 20 mm Hg from the right internal

jugular vein to perfuse the pulmonary artery of the isolated lung. A Transonic flow probe (Transonic Systems, Inc., Ithaca, N.Y.) was incorporated into the perfusion line for flow measurements. The isolated lung was suspended in a perfusion chamber, which was immersed in a 37° C water bath. Blood returned from the isolated lung was collected in the perfusion chamber. A roller pump returned the blood from the perfusion chamber to the left carotid artery of the host rat. The rate of blood returning to the host rat was adjusted to maintain satisfactory systemic pressure (Fig. 1). During the perfusion period, the isolated lung was ventilated with room air at respiratory rate of 40 to 50 rpm, tidal volume of 2.5 ml, and peak end-expiratory pressure of 0.5 cm H₂O.

After an initial 5- to 10-minute equilibration period, blood samples from the pulmonary artery and vein were taken simultaneously every 10 minutes for blood gas analysis and alveolar-arterial oxygen difference calculation with the IL BGE Blood Gas and Electrolytes Analyzer (Instrumentation Laboratories, Lexington, Mass.). Perfusion pressure, perfusion flow, airway pressure, and tidal volume were recorded continuously on a Gould multichannel recorder (Gould Inc, Centerville, Ohio). Using these parameters, we calculated pulmonary vascular resistance and airway resistance. At the end of the experiment, lung tissue samples were taken for wet/dry weight ratio measurements.

Animal groups studied. Twenty-four rats were used in this study. The rats were divided into three groups. Group 1 rats ($n = 8$) were used as normal controls. The left lungs were removed and immediately transferred to the perfusion apparatus for function studies. In this group, no interruption of ventilation occurred, and interruption of lung perfusion was routinely less than 10 to 20 seconds. Lung function in this group was assumed to be normal. In group 2 ($n = 8$), the left lungs were flushed with 10 to 15 ml of 4° C Euro-Collins solution at a gravity gradient of 20 cm H₂O and immersed in 4° C Euro-Collins solution for 24 hours. In group 3 ($n = 8$), the left lungs were also flushed with 10 to 15 ml of 4° C Euro-Collins solution, followed by 5 ml of 4° C saline solution containing DADLE (1 mg/kg, Peninsula Lab, Belmont, Calif.) and then immersed in 4° C Euro-Collins and DADLE solution for 24 hours. In groups 2 and 3, the bronchus was clamped at the end of inspiration to keep the lung inflated during storage.

Statistical analysis. Two-way analysis of variance was used for repeated measurements. If significance was established, the Student-Newman-Keuls test was used to analyze the difference between individual groups. A value of $p < 0.05$ was considered significant. All results were expressed as means $\pm$ standard error of the mean.

Results

Severe pulmonary edema and hemorrhage occurred in group 2 (Euro-Collins solution alone) within 30 minutes of perfusion, and no preparation could be perfused for more than 30 minutes because of occlusive pulmonary resistance. This phenome-

Fig. 1. Apparatus for isolated lung perfusion study. Venous blood from the host rat is used to perfuse the lung. Blood returned from the lung is infused into the carotid artery of the host rat. *PA*, Pulmonary artery; *PV*, pulmonary vein; *C*, perfusion chamber, *R*, reservoir.

non did not occur in either group 1 (control) or group 3 (Euro-Collins plus DADLE), where perfusion studies could be carried out for more than 60 minutes in these two groups. Oxygen tension and carbon dioxide tension in the pulmonary artery and vein of the isolated lung during the 60-minute reperfusion period remained stable and were comparable between groups 1 and 3. Pulmonary vascular resistance and airway resistance were also stable during the perfusion period in these two groups.

Blood flow and pulmonary vascular resistance of the isolated lung. Pulmonary blood flow ranged from 2.06 ± 0.25 to 3.50 ± 0.56 ml/min in group 1, from 0.65 ± 0.10 to 0.87 ± 0.10 ml/min in group 2, and from 1.68 ± 0.09 to 1.94 ± 0.21 ml/min in group 3. Pulmonary blood flow was lower in group 2 than in either group 1 or group 3. In group 1, pulmonary vascular resistance ranged from 7.96 ± 0.76 to 14.00 ± 2.60 mm Hg/ml/min; in group 2, it ranged from 33.00 ± 5.00 to 50.50 ± 11.00 mm Hg/ml/min ($p < 0.05$ as compared with group 1 and 3); and in group

3, it ranged from 13.60 ± 1.00 to 15.00 ± 1.00 mm Hg/ml/min. Because of high pulmonary vascular resistance in group 2, perfusion could not be continued after 30 minutes, indicating severe tissue edema and damage in this group. No difference was found in pulmonary vascular resistance between group 1 and group 3 (Fig. 2).

Airway pressure and resistance of the isolated lung. When tidal volume 2.5 ml was used, airway pressure ranged from 6.4 ± 0.4 to 9.3 ± 1.2 mm Hg in group 1, from 13.9 ± 0.9 to 17.6 ± 1.2 mm Hg in group 2, and from 9.3 ± 0.8 to 13.3 ± 1.2 mm Hg in group 3. Airway pressure was much higher in Group 2 ($p < 0.05$ as compared with group 1 and group 3). It was also higher after 20 minutes of perfusion in group 3 than in group 1 ($p < 0.05$).

Airway resistance ranged from 2.6 ± 0.2 to 3.4 ± 0.4 mm Hg/ml in group 1, from 5.5 ± 0.3 to 7.0 ± 0.4 mm Hg/ml in group 2, and from 3.8 ± 0.4 to 5.0 ± 0.5 mm Hg/ml in group 3. Airway resistance was much higher in group 2 than in groups 1 and 3 ($p <$

Fig. 2. Comparison of pulmonary vascular resistance (*PVR*) in the three groups.

Fig. 3. Comparison of airway resistance in the three groups.

0.05). However, airway resistance was also higher in group 3 than in group 1 after 20 minutes of perfusion ($p < 0.05$, Fig. 3).

Blood gases in the inflow and outflow of the isolated lung. Mean oxygen tension in the pulmonary artery (before oxygenation) was 33 ± 1.6 to 36 ± 2.1 mm Hg in group 1, 48 ± 5 to 52 ± 7 mm Hg in group 2, and 53 ± 4.0 to 60 ± 3.7 mm Hg in group 3. Mean

oxygen tensions from the pulmonary vein (after oxygenation by the isolated lung) were 130 ± 13 to 180 ± 11 mm Hg in group 1, 70 ± 9.0 to 92 ± 14 mm Hg in group 2, and 116 ± 8.0 to 173 ± 21 mm Hg in group 3. Oxygen tensions in group 2 were lower than in groups 1 and 3 during perfusion and worsened when perfusion time was longer, indicating impaired oxygenation capacity in this group (Fig. 4).

Fig. 4. Comparison of oxygen tensions in the isolated pulmonary vein in the three groups.

Carbon dioxide tension in the pulmonary artery was 30 ± 2.7 to 38 ± 2 mm Hg in group 1, 41 ± 4 to 41 ± 7 mm Hg in group 2, and 16 ± 2 to 23 ± 3 mm Hg in group 3. Carbon dioxide tension in the pulmonary vein was 17 ± 0.6 to 19 ± 2.0 mm Hg in group 1, 17 ± 2.5 to 23 ± 4 mm Hg in group 2, and 16 ± 2 to 23 ± 3 mm Hg in group 3. No statistically significant difference was found among the three groups, although perfusion time was much shorter in group 2.

Alveolar-arterial oxygen difference. Alveolar-arterial oxygen difference ranged from 4.68 ± 4.62 to 30.29 ± 10.28 mm Hg in group 1, from 44.01 ± 10.94 to 54.88 ± 6.16 mm Hg in group 2, and from 0.46 ± 0.46 to 23.49 ± 5.45 mm Hg in group 3. Alveolar-arterial oxygen difference was much higher in group 2 than either group 1 or group 3 ($p < 0.05$, Fig. 5).

Lung tissue wet/dry weight ratio after perfusion. Lung tissue wet/dry weight ratio after perfusion was 6.3 ± 0.4 in group 1, 8.7 ± 0.5 in group 2, and 5.7 ± 0.7 in group 3. Tissue wet/dry weight ratio was much higher in group 2 than in groups 1 and 3 ($p = 0.005$), even though the perfusion time was only 30 minutes in group 2. No difference was found in tissue wet/dry ratio between group 1 and group 3 (Fig. 6).

Discussion

The scarcity of suitable donor organs is the main limitation to transplantation. Among all the organs, the lung is probably the most difficult to preserve for several reasons: (1) the unique, delicate architecture

of the lung poses a special problem in preservation; methods allowing short-term storage of the kidney, liver, and heart have not been successful for lungs; (2) efforts to extend the ischemic period by cooling the lungs with cold gas ventilation, perfusion, or flushing have been ineffective, producing edema in preserved lungs; (3) as with the heart, total functional dependence is required of the preserved lung after it is transplanted, making the functional viability of tissue critical during a preservation period.⁴

Single-flush perfusion of the lungs is currently the most widely practiced technique in lung preservation. However, when simple flushing with various preservation solutions is performed, the results have generally been unsatisfactory, with only a few exceptions.⁵⁻⁷ For years, numerous chemicals and modifications, such as prostaglandins,^{8,9} oxygen free-radical scavengers,^{10,11} platelet-activating factor antagonists or leukocyte depletion,^{12,13} corticosteroids,¹⁴ and other chemicals,¹⁵⁻¹⁷ have been added to pulmonary flush solutions (e.g., modified Euro-Collins solution, University of Wisconsin solution, University of California-Los Angeles solution, Stanford solution, low potassium dextran solution, and Bretschneider's solution). Although various reports have indicated that such chemicals provide improved effects, of which prostaglandins are an excellent example, no additive has shown consistently reliable results.¹⁸⁻²² Our results from group 2 animals appear to agree with those of previous reports; that is, when preservation time exceeds 6 hours, lung

Fig. 5. Comparison of alveolar-arterial oxygen ($A-aO_2$) difference in the three groups.

Fig. 6. Lung tissue wet/dry ratios after preservation and perfusion in three groups.

function is severely damaged, as indicated by reduced perfusion flow, increased perfusion pressure, decreased oxygen tension in the pulmonary vein, and increased lung tissue water content. Euro-Collins solution alone is known to cause pulmonary

vasoconstriction. Although the other aforementioned solutions have been used by many centers in both animal and human models, no solution has been shown to be physiologically successful for safe human lung preservation beyond 6 hours.^{19, 23-26}

Fig. 7. Effect of DADLE on arterial blood pressure in an anesthetized dog.

Previous studies from our laboratory have shown that DADLE can effectively extend tissue survival time in a normothermic multiorgan preservation block. The mechanism by which DADLE extends tissue survival time in normothermic tissue preservation appears to be its ability to protect tissue from damage during various ischemic insults. The possible mechanisms that might be responsible for tissue protection are as follows: (1) reducing tissue metabolism during ischemia; (2) reducing or eliminating liver congestion; (3) improving tissue perfusion; and (4) reducing platelet/leukocyte aggregation.^{1, 27} Of these properties, the elimination of liver congestion and the reduction of platelet/leukocyte aggregation can only be seen during normothermic perfusion. However, if DADLE can reduce tissue metabolism or improve tissue perfusion, it would also protect tissue from ischemic damage during hypothermia. If this is the case, the potential use of this chemical will be tremendous. Not only will it improve organ preservation by adding positive effects to presently used preservation solutions, but it will also enhance the effectiveness of various currently used cardioplegic solutions and possibly that of other hypothermic treatment modalities.

Our results indicate that, when DADLE was added to the Euro-Collins solution, lungs that had been preserved for 24 hours maintained excellent function, a result that has not been achieved in

previous reports that used Euro-Collins solution alone. The most noteworthy findings in these studies were the near-normal tissue wet/dry weight ratio along with near normal pulmonary blood flow, pulmonary vascular resistance, and good oxygen-exchanging capacity in the lungs after 24 hours of preservation. In comparison with normal lungs (without preservation), airway resistance in the lungs preserved with DADLE increased only slightly after preservation. The resistance was much lower than that measured in lungs preserved with Euro-Collins solution alone.

Because of the complexity of opioids and their receptors, studies to date have primarily concentrated on the reactions of different opioids with different receptors. The effects of opioids on the cardiovascular system have been studied for many years, and the results are controversial.^{28, 29} Through a MEDLINE search, we found no reports that related opioid agonists to tissue metabolism or organ preservation other than those that have been published from our laboratory.^{1, 27} On the other hand, opioid antagonists, such as naloxone and nalmefene, have been reported to have a tissue protective effect on liver, kidney, and intestine during ischemic insult. This effect is believed to be related to the ability of opioid antagonists to scavenge superoxide.³⁰⁻³²

Several possibilities exist for the protective effect

of opioid agonists such as DADLE on tissue damage from ischemia. The most intriguing of these possibilities relates to a reduction in tissue metabolism. Previous studies have indicated that delta opioid receptors may be responsible for naturally occurring animal hibernation. However, not all the opioid subtypes are involved in hibernation. Infusing morphine, a μ opioid agonist, into summer-active ground squirrels caused an insignificant bout of hibernation of extremely short duration. Similar results of short bouts of hibernation have been obtained with another peptidic μ opioid, morphiceptin, a naturally occurring k peptide of brain origin, dynorphin A, and a highly selective synthetic k opioid, U69593. On the other hand, animals receiving implanted pumps containing DADLE began hibernating shortly after implantation and infusion. Continuous subcutaneous administration of naloxone, an opioid antagonist, diminished the frequency and length of hibernation bouts in *Citellus tridecemlineatus*.^{33, 34} These results strongly suggested that the μ opioid receptor and its ligand are intimately involved in the induction of animal hibernation, and animal hibernation may be induced by a mechanism involving an endogenous opioid that acts through binding to receptor sites.³⁵ Thus, it is possible that DADLE protects tissue from various types of damage during ischemia by a mechanism similar to animal hibernation. Inhibition of tissue metabolism or improvement of microcirculation by means of vasodilation probably plays an important role in this protection. We have shown, in previous experiments, a severe reduction of arterial blood pressure after intravenous injection of DADLE into anesthetized animals (Fig. 7). We have not yet performed systemic toxicity tests for DADLE. However, in our multiorgan preservation studies we did not find a toxic effect on any major organ.¹

Evaluation of lung function after preservation has been hampered by the lack of a reliable testing model. In this study, the viability of the lungs was tested by means of a living rat perfusion model. Venous blood from the host rat perfused the isolated lung. Blood returned from the isolated lung was pumped back into the arterial system of the host. This route reduced the load on the host heart, and the aggregates and particles returned from the isolated lung were filtered by the host's peripheral microvasculature. In this setting, we were able to use one host rat for two to three isolated lung studies totaling five or more hours of perfusion. This model has proved to be simple and stable, yet sensitive for

testing isolated lung function. Although it is not always advisable to extrapolate experimental results from tests on small animals to large animals or to human beings, such animal experimentation provides an effective and economical way to screen drugs and solutions for hypothermic lung preservation.

In summary, the present study clearly shows, for the first time, that DADLE has a positive effect on hypothermic preservation of rat lungs. This effect is related to the ability of DADLE to protect tissue from various forms of damage during ischemia. The exact mechanism of this effect is not clear and deserves further study.

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