A system for pharmacological studies in small animals at high pressure

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Aanderud L. A system for pharmacological studies in small animals at high pressure. Undersea Biomed Res. 1981; 8(2):69–74. A four-liter pressure chamber was constructed for pharmacological experiments in rats; it consisted of the following: 1) a system for repeated, accurate injection; 2) an alternative single-dose injection system; 3) a system for direct blood sampling to the outside. The injection and sampling systems were tested for possible drug adsorption at 71 ATA. The accuracy of drug concentration measurements in samples collected through the sampling system at pressure was found to be satisfactory.

- hyperbaric chamber
- pharmacology
- animal chamber
- injection system
- sampling system
- catheter implantation

It has been shown that the effects of several drugs are modified by high pressure in laboratory animals (1–5) and in the human being (6, 7). Investigations in living animals have been limited to pharmacodynamic experiments (for review see reference 8) and drug toxicity studies (9–11), and it is not known whether high pressure causes changes in drug distribution, metabolism, or excretion.

Pharmacokinetic experiments with living animals at high pressure that involve drug administration and blood sampling necessitate certain modifications to an ordinary animal pressure chamber. An ingenious system constructed by Obrenovitch and Brue (12) permits repeated blood sampling into cooled tubes that can be decompressed independently of the animal. This apparatus, however, requires a considerable amount of engineering and chamber space. D’Aoust and Swanson (13) have developed a procedure for bubble-free decompression of blood samples that uses volumetric dilution with gas-free saline before decompression. This method requires handling inside the chamber and transport of each sample through the chamber lock.

The present report describes a method for pharmacological studies at high pressure; it consists of a pressure chamber with two different injection systems and a sampling system for whole blood. This apparatus has been specifically designed for studies of drug kinetics and drug effects in small experimental animals. A series of in vivo and in vitro experiments was undertaken to test the systems.
MATERIALS AND METHODS

Chamber

A four-liter cylindrical steel pressure chamber was designed for a working pressure of 150 ATA (Fig. 1); the figure legend identifies the numbered parts of the chamber. One end consisted of a 7-cm-thick acrylic circular viewport. The other end was equipped with a 12-pin Brantner plug (XSL-12-CCP, Brantner & Co., Ltd., Newport Beach, CA), which was used for power supply and monitoring. A fan for gas mixing was driven by an induction motor. The rectal and atmospheric temperatures were monitored by two integrated circuit temperature transducers (AD 590, Analog Devices, Inc., Norwood, MA) with a precision of ± 0.1°C, and displayed on a multimeter (Data Precision Corp., Wakefield, MA). Temperature control (33°C – 35°C) was achieved by electrical heating, using an external coil and taking into account the adiabatic heat produced during compression. Arterial blood pressure was monitored by connecting the dome of a pressure transducer (AE 480, A/S Mikroelektronikk, Horten, Norway) to an extension in the arterial blood sampling system, and it was displayed on a pen recorder and an oscilloscope. The chamber pressure was read on a Roylyn precision manometer (3-D-Instruments, Huntington Beach, CA).

Chamber atmosphere

The atmosphere within the pressure chamber was cleared of CO₂ with moistened soda lime, which was spread out in a tray covering the bottom of the chamber. The amount of soda lime used was based on the known absorbing capacity, the animal weight, and the duration of the experiment. The partial pressure of CO₂ was monitored by CO₂ test tubes (Drägerwerke, Lübeck, Germany), with a detection limit 0.01% v/v. The partial pressure of oxygen was monitored with a paramagnetic oxygen analyzer (Servomex DA 580, Taylor & Servomex, Ltd., Crawbrough, Sussex, England).

Drug injection system

Two alternative systems that were used for administering the drugs are shown in Fig. 1. The drug-containing solution, which had been injected into the sample loop of a high pressure

Fig. 1. Outline of the pressure chamber. 1, Heating coil; 2, acrylic viewport; 3, drug injection syringe with one loose piston, which is moved with fluid from the outside pump; 16, 4, induction motor; 5, fan with wire net cage; 6, 12-pin plug; 7, gas outlet valve; 8, gas inlet valve; 9, safety valve; 10, blood sampling syringe; 11, blood sampling miniature needle valve; 12, pressure gage; 13, gas supply valve; 14, high pressure liquid chromatograph injector; 15, 3-way valve; 16, manual pressure pump; 17, water-containing syringe for refilling of the pressure pump, 16.
PHARMACOLOGICAL STUDIES AT HIGH PRESSURE

liquid chromatograph sample injector (Model 7120, Rheodyne, Inc., Berkeley, CA), was transferred via stainless steel tubing through the chamber port by application of hydraulic pressure with a saline-filled manual pump. Inside the chamber a polyethylene catheter (PE-50, Intramedic, Clay Adams, Parsippany, NJ), which had previously been inserted into the femoral vein of the experimental animal, was connected to the tubing. This system permitted repeated injections of one or more drugs from the outside.

A simpler procedure was used in experiments where only a single injection was required. The volume of drug solution to be injected was transferred to a disposable syringe that was fitted with a piston and was placed inside the chamber as shown in Fig. 1. Behind this piston was a fixed cylinder end plate through which the steel tubing from the pressure pump penetrated. The space between the pistons was increased by application of hydraulic pressure with the manual pump, and the drug was injected through the catheter that was connected to the syringe.

Sampling system

The polyethylene catheter, previously inserted into the femoral artery, was led through a brass tubing to the outside, where it was connected to a miniature needle valve (Hoke Milli-Mite Forged Metering Valve No. 1345 G4Y, Hoke, Inc., Cresskill, NJ). The needle was then screwed into the brass tubing. To reduce the dead space in the needle valve a brass plug, which was penetrated by a needle to permit connection to the catheter, was lodged in the lumen. The other port of the valve was drilled out to fit a disposable 1-ml syringe. After the dead space in the system (0.15 ml) was discharged, a syringe was filled via the valve to contain approximately 0.3 ml of blood and gas bubbles from the pressurized animal (approximately 200 mg hemolyzed blood). The syringe was weighed with its contents and then reweighed after being emptied into a small test tube, to assess the weight of the sample. All parts of the tubing and valve were pretreated with heparin (5000 U/ml) and a 1% silicone solution (Siliclad, Clay Adams, Parsippany, NJ) to prevent clotting.

Animal preparation

Male Wistar rats weighing 200–300 g were anesthetized with a combination of fluanisone and fentanyl, 10 and 0.2 mg/ml, respectively, 0.05 ml/kg subcutaneously (Hypnorm®, Mekos, Hälsingborg, Sweden), Polyethylene catheters (PE-50) were inserted into the femoral vein and femoral artery and led subcutaneously to the flank. To ensure adequate hydration during the experiment, 3 ml of isotonic saline were given subcutaneously, and 2 ml were injected through the catheters with 200 U of heparin. The animals were then kept in restraining cages made to fit the pressure chamber, and they had free access to pellets during the experiments.

Compression procedure

The chamber was pressurized with air to 4 ATA, and from then on pure helium was used, proceeding at a compression rate of 1 ATA per min. After reaching 71 ATA, the rats were allowed one hour to adapt to the pressure before the pharmacological experiments began. Oxygen was added as necessary to keep its partial pressure between 0.4 and 0.6 ATA after two hours. The temperature, CO₂ content, and O₂ partial pressures were monitored as described. The animals were killed by rapid decompression after the experiments.
Testing of drug injection and sampling systems

The systems were tested by use of [5-14C]diazepam (Hoffman-La Roche & Co., Basel, Switzerland) having a specific radioactivity of 186 μCi/mg and diluted with unlabeled diazepam.

Injection system. The drug content in the injection syringe (116.8 μg/ml with a specific activity of 8.37 μCi/mg) was measured immediately after instillation in the syringe, and again after two hours, to ensure that no adsorption of the drug occurred before the experiments.

Sampling system. For testing of the sampling system for possible drug adsorption, a tube containing plasma with 4.71 μg/ml diazepam (specific activity: 8.37 μCi/mg) was placed in the pressure chamber and connected to the sampling system. Aliquots (100 μl) were collected immediately through capillary tubes. After one hour at 71 ATA a new series was collected through the polyethylene tubing, needle valve, and disposable syringe, as described. These were weighed and transferred to the counting vials. A final series of samples were taken with capillary tubes after decompression.

Control experiments in vivo

The design of the pharmacological studies involves crossover experiments with blood sampling on two consecutive days, Day 1 at 1 ATA and Day 2 at 71 ATA. This design was followed for a group of test animals (n = 9). The total blood loss by sampling during the two days amounted to approximately 3.5 ml.

Analytical methods

The [5-14C]-diazepam was measured by liquid scintillation counting. Since the blood samples were partially hemolyzed during the rapid decompression, all blood samples were frozen to obtain complete hemolysis before the analyses were performed.

RESULTS

Injection system

The diazepam concentrations in the injection syringe were initially 116.8 ± 1.74 μg/ml (mean ± SD, n = 5), and after 2 h 112.1 ± 3.19 μg/ml.

Sampling system

The test plasma was found to contain 4.71 ± 0.043 μg/ml (n = 5) and 4.55 ± 0.066 μg/ml (n = 5) before compression and after decompression, respectively, when blood was collected into glass capillary tubes. When it was collected into plastic syringes, the values were 4.55 ± 0.082 μg/ml (n = 5) before compression and 4.52 ± 0.086 at pressure.

DISCUSSION

Pharmacological experiments in vivo at high pressure entail technical and practical problems regarding drug injection, blood sampling, and animal preparation.
The high pressure liquid chromatograph injector described in this report is simple and reliable, and it permits accurate repeated administration of one or more drugs. Injection loops with volumes ranging from 10 μl to 2 ml are available. Since these do not have to be filled completely, any volume within this range may be injected. The alternative, simpler system, with a disposable syringe inside the chamber, was designed for single injections at pressure. The drug solution is instilled in the syringe before pressurization and is kept there until the injection takes place. Since this latter system does not permit flushing through the catheter after the injection, a very small part of the drug will remain in the catheter. All control experiments should be carried out with the identical technique, or the dose should be corrected to take this loss into account. No adsorption of the drug diazepam took place in the injection system. The present method of blood sampling is simpler than those used in earlier studies of biochemical parameters and blood gases (12, 13).

The concentration of diazepam in the samples collected at high pressure was slightly lower (less than 5%) than in those collected through capillary glass tubes before pressurization. Compared with samples collected directly into plastic syringes before compression, however, no difference was found. No drug adsorption to the tubing or valve took place, and no error was introduced by the rapid decompression.

The animals had the catheters inserted into the femoral artery and vein of the same leg. The leg was rendered ischemic and paretic and was probably painful. The catheters penetrated the skin in the flank in order for them to be accessible, and this system necessitated the use of a restraining cage.

The site of the catheter implantation is of importance. In more recent experiments the tail artery and the femoral vein have been used, and the catheters have been coiled up in a subcutaneous pocket on the back. This pocket can easily be opened by removing two skin sutures. When this technique is used, the rats do not have to be restrained between the experiments.

Preliminary experiments performed with rats with intact eardrums, and with rats on which paracentesis had been performed, showed no difference with respect to blood pressure and heart rate variations. Rats with intact eardrums, however, appeared to be more distressed during compression. Therefore, it seems wise to perforate the eardrums before the experiments, and this is very easily accomplished in the anesthetized animal with a small injection needle. Later experience has also shown that preparative handling of the animals, to make them accustomed to the restraining cage and the pressure chamber, calms the animals considerably.

The best type of restraining cage seems to be a transparent Plexiglas tube, slightly bigger than the animal, with sufficient perforations for heat exchange and with an open front. The rats seem most comfortable when they are able to stick their heads out. Two loose skin sutures in the sides prevent the animals from escaping.

The success rate of the described procedures depends on a number of factors of technical and practical nature. In our hands 50% to 70% of the experiments are successful. The most common causes of failure have been catheter malfunction because of thrombus formation, perforation of blood vessels, and catheter dislocation.
Aanderud L. Système d'études pharmacologiques à haute pression sur les petits animaux. Undersea Biomed Res 1981; 8(2):69–74.—Une chambre à pression de quatre litres a été construite pour l'expérimentation pharmacologique des rats; elle consistait en ce qui suit: 1) un système pour une injection répétée et précise; 2) un système alternatif pour injection à dose unique; 3) un système de prélèvement de sang lié directement à l'extérieur. Les systèmes d'injection et de prélèvement ont été testé pour absorption possible de produits narcotiques à 71 ATA. L'exactitude des mesures de concentrations de produits narcotiques des échantillons prélèves à travers le système de prélèvement a été jugée satisfaisante.

chambre hyperbare
pharmacologie

système d'injection
système de prélèvement

REFERENCES