A method for the collection of end-tidal gas samples from small animals

A.S. CLANACHAN, J.S. GILLESPIE, R.A. MILLAR & T.C. MUIR

University Department of Anaesthesia, Glasgow Royal Infirmary and Department of Pharmacology, Glasgow University, Scotland

The collection of end-tidal gas samples has been carried out successfully in larger animals including human subjects (Rahn & Otis, 1949), but the procedure is more difficult in small species due to the low tidal volumes and high respiratory rates. While samples might be obtained from rapidly-breathing small animals by fast manual withdrawal (White, Johnston & Eger, 1974), this is subject to operator error.

A semi-automated end-tidal gas sample collector is demonstrated for use with tracheostomized pithed rats (Gillespie & Muir, 1967), mechanically ventilated at 60/minute. It includes a sampling pump (Watson Marlow, M.H.R.E.) which is operated for 200 ms during each respiratory cycle. In this period a sample (about 0.3 ml) is withdrawn automatically from the tracheal cannula into a 10 ml glass syringe. The pump is controlled by a timer (Devices Digitimer 3840) which is triggered from the positive inspiratory pressure by means of a simple diaphragm pressure switch. The withdrawal of end-tidal samples, is achieved by introducing a timed delay between inspiration and the sample period of the pump. Subsequent analysis of the carbon-dioxide concentration verified the end-tidal nature of the sample. The collection of about 30 samples provides an adequate volume for subsequent gas analysis.

The method, which may be applied to other small animals such as guinea-pigs and rabbits, has been used to measure alveolar anaesthetic concentrations.

References

GILLESPIE, J.S. & MUIR, T.C. (1967). A method of stimulating the complete sympathetic outflow from the spinal cord to the blood vessels in the pithed rat. *Br. J. Pharmac. Chemother.*, **30**, 78-87.

RAHN, H. & OTIS, A.B. (1949). Continuous analysis of alveolar gas composition during work, hyperpnea, hypercapnia and anoxia. *J. appl. Physiol.*, **1**, 717-724.

WHITE, P.F., JOHNSTON, R.R. & EGER, E.I. (1974). Determination of anesthetic requirement in rats. *Anesthesiology*, **40**, 52-57.

The quantitative assay of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ in biological extracts using a Finnigan 3000D quadrupole mass spectrometer

C.N. HENSBY & B. NAYLOR (introduced by E.W. HORTON)

Department of Pharmacology, University of Edinburgh

The use of a multiple ion detection system that enables the quantitative assay of prostaglandins E_2 and $F_{2\alpha}$ either separately or in parallel is described. The method is based on the addition of a known amount of the corresponding d_4 deuterium isotopes to the biological samples immediately before extraction.

The ratio of protium to deuterium peaks (after derivatization) are then obtained by means of an accelerating voltage alternator. A calibration curve is obtained by plotting the ratio of protium to deuterium peak heights over the range 1 to 1,000 ng of protium.

The PGE_2 and $PGF_{2\alpha}$ are prepared as the methyl ester/methoxime/TMS and methyl ester/TMS derivatives respectively as described previously (Thompson, Los & Horton, 1970; Blatchley, Donovan, Horton & Poyser, 1972). These derivatives produce ions at m/e of 295 (M − (199 + 45)) for PGE_2 and 423 (M − (90 + 71)) for $PGF_{2\alpha}$. The corresponding deuterium ions are at m/e values of 299 and 427.

This method has been compared in two instances with results obtained by alternative methods and found to be in good agreement.

In the first example adult virgin female guinea-pigs were killed on day 7 of the cycle and the uteri removed and weighed. The uteri were homogenized and incubated, and the prostaglandins produced were extracted by solvents and purified by silicic acid column chromatography as described previously (Poyser, 1972). After chromatography the samples containing $PGF_{2\alpha}$ were assayed biologically on the rat fundal strip or by GCMS as described above. The amounts of $PGF_{2\alpha}$ produced (ng/100 mg tissue ± s.e. mean) were 45.8 ± 3.4 ($n = 5$) by bioassay, and

53.7 ± 3.7 (n = 5) by GCMS. This shows that the two methods give comparable results.

In the second example the effect of NADH (2 mM) on the metabolism of prostaglandin E_2 by the 9-oxo-reductase, present in sheep blood has been studied. Previously this has been shown not to stimulate the reduction of PGE_2 to $PGF_{2\alpha}$ by the enzyme (Hensby, 1974). The rates of $PGF_{2\alpha}$ production in the presence and absence of NADH were found to be comparable when quantitatively assayed either by GCMS or tritium isotope analysis.

The Finnigan 3000D was purchased with a grant from the Medical Research Council.

References

BLATCHLEY, F.R., DONOVAN, B.T., HORTON, E.W. & POYSER, N.L. (1972). The release of prostaglandins and progestin into the utero-ovarian venous blood of guinea-pigs during the oestrous cycle and following oestrogen treatment. *J. Physiol.*, **223**, 69-88.

HENSBY, C.N. (1974). Reduction of prostaglandin E_2 to prostaglandin $F_{2\alpha}$ by an enzyme in sheep blood. *Biochim. Biophys. Acta* (in press).

POYSER, N.L. (1972). Production of prostaglandins by the guinea-pig uterus. *J. Endocr.*, **54**, 147-159.

THOMPSON, C.J., LOS, M. & HORTON, E.W. (1970). The separation, identification and estimation of prostaglandins in nanogram quantities by combined gas chromatography-mass spectrometry. *Life Sciences*, **9**, 1, 983-988.

A novel method for evaluating anti-inflammatory drugs in the conscious guinea pig

WENDY J. McDONALD-GIBSON & C. SCHNEIDER

Research Department, Miles Laboratories Limited, Stoke Court, Stoke Poges, Slough SL2 4LY, England

The dorsal surface of each ear of individually marked guinea pigs of 200-300 g body-weight was depilated by application of Nair cream. After removal of the cream by washing in tap water the animals were placed in their home cages. Twenty hours later they were placed four in a cage, separated from each other by wire barriers. Test substances were applied in 100-150 mg of a water miscible cream (Acid Mantle cream) to one ear of each animal, the other ear being treated with vehicle.

Twenty minutes after each application all four guinea pigs were placed under a 20 watt 57 cm long ultra-violet strip light placed 8 cm above their heads for 30 minutes. In unprotected animals, such irradiation was sufficient to cause marked increase in temperature of the ears, erythema, delayed oedema and blister formation. During experiments, no food or water was given to the animals until observations on ear temperatures were complete, usually 4 h after irradiation.

Substances applied prior to irradiation of the guinea pigs were re-applied after irradiation. Some substances were only applied after irradiation.

Substances in a concentration of up to 10% w/w applied to the ears included the following: para aminobenzoic acid (PABA), propyl gallate, promethazine hydrochloride, uval (2-hydroxy-4-methoxy-benzophenone-5-sulphonic acid) and bufexamac [p-(n-butoxy)phenylacethydroxamic acid)]. In some experiments propyl gallate or bufexamac was applied only after irradiation.

The sodium salts of aspirin or indomethacin were given in aqueous solution by subcutaneous injection 30 min prior to irradiation of the animals. In one experiment two subsequent treatments with sodium aspirin were given 2 h and 4 h after the first.

Erythema was assessed subjectively by two observers.

Pyrexia was estimated from 5-280 min after irradiation by placing a thermometer probe set in plasticine firmly on the dorsal surface of each ear in turn for 2.5 minutes. Temperature (°C) was measured via a Wheatstone bridge to a twin channel Devices polygraph using a DC2-D pre-amplifier.

Ear thickness was measured by a micrometer screw gauge immediately prior to application of test substance and 24 h after irradiation. Oedema was taken as being proportional to the increase in ear thickness.

Blister formation was assessed subjectively by two independent observers from 5-7 days after irradiation.

Erythema and pyresis were inhibited by prior treatment with subcutaneous sodium aspirin (150 mg/kg) or by topically applying adrenaline, PABA, propyl gallate, promethazine, bufexamac or Uval prior to irradiation or by applying propyl gallate after irradiation. PABA, propyl gallate and promethazine also inhibited oedema. Propyl gallate applied after irradiation gave some protection against blister formation.