Biochemical Pharmacology, Vol. 38, No. 16, pp. 2745–2748, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00
Maxwell Pergamon Macmillan plc

Anaesthetic influences on brain haemodynamics in the rat and their significance to biochemical, neuropharmacological and drug disposition studies

(*Received* 15 *December* 1988; *accepted* 24 *February* 1989)

Many experiments, in the small laboratory animal, are conducted under the influence of anaesthesia. Deleterious effects of general anaesthesia upon cardiovascular and haemodynamic status have been recognized [1] and in particular, anaesthetic-induced alterations in brain blood flow may be of significance in the performance of neuropharmacological studies. For a highly lipid soluble compound, whose transport across the blood–brain barrier does not represent a rate limiting step, changes in blood flow may affect the rate of brain uptake. Further, it has been postulated [2] that central ischaemic actions might influence neuronal homeostasis. However, limited attention has been placed upon the differential influence that some anaesthetic regimens may have upon brain haemodynamics.

The aim of the present investigation was to examine, in the acute surgically-prepared rat, the effects that a range of commonly employed, parenterally administered general anaesthetic regimens may have upon brain perfusion. To this end, the reference blood sample microsphere technique [3] has been employed since it readily allows the simultaneous assessment of both cardiac output and regional haemodynamics.

Materials and methods

Anaesthetic regimens examined. This study was performed using male Wistar rats (270 ± 19 g) that were allowed free access to water until the time of the experiment, and laboratory rat chow (Grain Harvesters Ltd., U.K.) until 4 hr prior to the experiment.

The anaesthetic regimens were:

(1) [H] — Fentanyl and fluanisone mixture ("Hypnorm"; Janssen Pharmaceuticals, U.K.); 0.26 and 8.3 mg/kg i.p., respectively) given in combination with midazolam ("Hypnovel"; Roche Pharmaceuticals, Welwyn Garden City, U.K.); 4.16 mg/kg i.p.) for induction of anaesthesia. Fentanyl and fluanisone (0.08 and 2.50 mg/kg i.p.) were subsequently given every 30 min for maintenance.

(2) [U] — Urethane (Sigma Chemical Co., Poole, U.K.; 1.75 g/kg i.p.) given as a 25% w/v solution in 0.9% w/v saline.

(3) [P] — Pentobarbitone sodium (Sigma; 67 mg/kg i.p.) for induction of anaesthesia given as a 1.5% w/v solution in 0.9% w/v saline. Pentobarbitone sodium (6–7 mg/kg i.p.) was subsequently given every 60 min as a maintenance dose.

(4) [K] — Ketamine ('Vetalar'; Parke Davis Pharmaceuticals, Pontypool, U.K.; 80 mg/kg i.p.) given in combination with midazolam ("Hypnovel"; Roche Pharmaceuticals; 5 mg/kg i.p.) for induction of anaesthesia. Ketamine (20 mg/kg i.p.) was subsequently given every 30 min for maintenance.

(5) [S] — Alphaxalone and alphadolone mixture ("Saffan"; Glaxovet Ltd., Harefield, U.K.; 9 and 3 mg/kg i.v., respectively) for induction of anaesthesia injected via the penile or caudal vain. Alphaxalone and alphadolone (3 and 1 mg/kg i.v.) were subsequently given every 15 min for maintenance.

The anaesthetic doses used in this study were the minimum required to produce surgical anaesthesia and are within the dose ranges commonly employed for laboratory anaesthesia in the rat.

Assessment of the distribution of cardiac output. The assessment of regional haemodynamics was performed using the microsphere technique of McDevitt and Nies [3]. The technique relies upon organ capillary trapping from a left intra-ventricular injection of radiolabelled microspheres, simultaneous withdrawal (at a constant and known rate) and collection of a reference aortic blood sample.

Once the animals had reached a sufficient depth of anaesthesia the right carotid artery was exposed, separated from the vagus nerve and catheterized (polythene tubing; i.d. 0.58 mm; o.d. 0.96 mm; code 800/100/200/100, Portex, U.K.). With the aid of pressure monitoring the tip of the catheter was manipulated into the left ventricle.

Blood pressure measurements were made prior to and immediately following (for a period of 90 sec) microsphere injection, using an intra-arterial method with a catheter placed in the right femoral artery (polythene tubing; code 800/100/200/100, Portex, U.K.). The catheter was connected via a fixed volume pressure transducer (no. 3552CA, Ormed Ltd., U.K.) to an MX 2 Devices recorder (Ormed Ltd., U.K) with internal calibration.

The protocol consisted of a 60 min period of stable anaesthesia, after surgical preparation was complete, before the microspheres were injected. A dose of approximately 1.99 μCi or 60,000–80,000 ^{113}Sn(tin)-labelled microsphe (15 ± 1.5 μm; stored in physiological saline, code NEM-062A; Nen-Trac, Dupont Ltd., Stevenage, U.K.; sp. act. 63 dpm/microsphere) was injected into the left ventricle over a 5 sec period, in 0.3 ml of 1 0.01% v/v Tween 80/0.9% w/v saline solution. The microsphere injection was immediately followed by 0.3 ml of 0.9% w/v saline injection wash. Simultaneously with microsphere injection, a reference blood sample was withdrawn from a left femoral artery catheter (polythene tubing; code 800/100/200/100, Portex U.K.) at a constant withdrawal rate of 0.43 ml/min (modified slow infusion pump; Scientific and Research Instruments Ltd, U.K.) and for a 90 sec period following microsphere injection.

To maintain catheter patency, the left femoral catheter contained 500 I.U./ml of sodium heparin (Sigma) /0.9% w/v saline. The right carotid and right femoral artery catheters contained 100 I.U./ml sodium heparin/0.9% w/v saline.

All surgical wounds were covered with gauze kept moist with 0.9 w/v saline to minimize tissue fluid loss. Rectal temperatures were monitored and maintained at 38 ± 1° using an incandescent lamp and heated surgical tray. Tracheostomies were performed as an aid to respiration throughout the period of anaesthesia.

Animals were killed at termination of the experiment by injection of air into the left ventricle, the organs were removed, washed in 0.9% w/v saline and blotted dry. The alimentary tract was cleared of undigested material and the organ weights recorded.

Organs removed included heart, lungs, right and left kidneys, brain, hepatosplanchnic tissue (spleen, stomach, pancreas and mesentery), small intestine, large intestine, and the liver.

The organs, together with the reference blood sample, were analysed radiochemically using an LKB 1275 Minigamma counter (window setting, 100–450 keV; counting efficiency for ^{113}Sn-20%).

Calculation of the distribution of cardiac output. Cardiac output and regional blood flows were calculated as described by McDevitt and Nies [3], using a computer program (Dr. C. R. Hiley, University of Cambridge) running on a BBC Master series microcomputer. The requirements used in validating the success of the microsphere technique included the following.

(1) Neither the injection of microspheres nor the collection of the reference blood sample should alter haemodynamics. This was validated to some extent by ensuring that no change in mean arterial pressure occurred following microsphere injection.

(2) Efficient mixing of the microspheres in the proximal aorta is essential and relies upon correct placement of the catheter tip in the left ventricle. This was validated by less than 15% of the injected microsphere dose remaining in the heart, and there existing a difference of less than 10% in microsphere trapping between right and left kidneys.

(3) The percentage of microspheres not trapped by the organs on a single pass and passing through arteriovenous shunts must be small. This was validated by less than 3% of the injected microsphere dose being found in the lungs.

(4) The dose was chosen to ensure that a minimum of 400 microspheres were distributed to each organ examined.

Statistical analysis was accomplished by one-way analysis of variance and Duncan's multiple range test.

Results

Cardiac output. It was notable that "Hypnorm/Hypnovel" anaesthesia resulted in a cardiac output significantly greater (32–85%) than all other anaesthetic regimens. In the case of "Saffan" anaesthesia cardiac output was significantly greater (26–39%) compared to ketamine/"Hypnovel" or urethane anaesthesia. The rank order of anaesthetic effects upon cardiac output is shown in Fig. 1.

The validity of our microsphere technique may be substantiated by reference to previously published results obtained, using the microsphere technique, in pentobarbitone anaesthetized rats. Our data closely agree with some reported literature values for cardiac output ranging from 20.8 to 25.3 ml/min/100 g body weight [3–6].

Distribution of cardiac output to the brain. The distribution of cardiac output to the brain, with ketamine/"Hypnovel" anaesthesia, was significantly greater (52–128%) compared to all other anaesthetic regimens. Brain distribution of cardiac output with pentobarbitone, urethane or "Saffan" anaesthesia was significantly greater (35–50%) compared to "Hypnorm/Hypnovel" anaesthesia (see Fig. 1).

Brain blood flow. Ketamine/"Hypnovel" anaesthesia resulted in a brain blood flow significantly greater (36–85%) than either "Saffan", pentobarbitone or urethane anaesthesia. Blood flow with "Hynorm/Hypnovel", "Saffan" or pentobarbitone anaesthesia was significantly greater (38–60%) compared to urethane anaesthesia (see Fig. 1). The correlation between anaesthetic-induced changes in cardiac output and brain blood flow was not significant ($P > 0.05$; $r = -0.15$).

Mean arterial pressure. The mean arterial pressures (results: mean ± SD) were as follows: Urethane 90 ± 6 mmHg; "Hypnorm/Hypnovel" 104 ± 6 mmHg; Pentobarbitone 128 ± 8 mmHg; "Saffan" 116 ± 4 mmHg; Ketamine/"Hypnovel" 126 ± 8 mmHg. Thus, mean arterial pressures did not alter between pre- and post-microsphere injection. In addition, the mean microsphere count remaining in the heart was 5.8%, and that in the lungs 1.9% of the injected i.v. dose, while the mean difference in microsphere counts between left and right kidney was 7.7%.

Discussion

The differential influence of anaesthetic regimens upon brain blood flow would appear to be maximal for ketamine/"Hypnovel" and urethane anaesthetic regimens. Urethane anaesthesia resulted in a brain blood flow that was approximately 54% of that obtained with ketamine/"Hypnovel". The percentage distribution of cardiac output to the brain was significantly greater in the ketamine/"Hypnovel" anaesthetized animals compared to all other anaesthetic regimens.

Idvall and co-workers [7, 8] reported a significant increase in the percentage distribution of cardiac output to the brain in rats anaesthetized with ketamine, a value of 2.6% being recorded, compared with 2.1% in chronically catheterized conscious animals. In the present study ketamine/"Hypnovel" anaesthesia resulted in a value for the percentage distribution of cardiac output to the brain of 2.5%. Dawson *et al.* [9], who studied cerebral blood flow in dogs, and Takeshita *et al.* [10] who made similar measurements in man, both observed that ketamine anaesthesia yielded an increase in the fraction of cardiac output going to the brain. They concluded that this was the result of cerebral vasodilatation. Ketamine anaesthesia, on the other hand, was observed not to induce a change in cerebral metabolism.

That the brain blood flow in our ketamine/"Hypnovel" anaesthetized animals remains reduced compared to previously reported brain blood flow values in the conscious rat (1.0 ml/min/g tissue) [7, 8] reflects the detrimental influence of the ketamine/"Hypnovel" anaesthetic regimen upon cardiac output.

Ketamine anaesthesia is usually not associated with cardiovascular depression. Indeed, cardiovascular response to ketamine anaesthesia is often characterized by increased cardiac output [8, 11]. In this study the ketamine dose administered (80 mg/kg i.p.) to produce surgical anaesthesia was considerably greater than comparative initial doses that have previously been employed to produce anaesthesia in the rat (30 mg/kg i.v.) [8] or in man (2 mg/kg i.v.) [7]. Recently Chen *et al.* [12] have demonstrated, in dogs, the dependence of ketamine-induced cardiovascular effects upon dosage. Profound cardiovascular depressor responses were observed with doses greater than 20 mg/kg i.v. Similarly Diaz *et al.* [13] demonstrated that ketamine anaesthesia can result in significant dose-dependent decreases in canine myocardial contractility. Haskins *et al.* [14] have demonstrated that ketamine administration, in conjunction with diazepam, was associated with less cardiovascular stimulation than when ketamine is administered alone.

In the present investigation the total ketamine dose administered in conjunction with the coadministration of a benzodiazepine may explain the relatively low cardiac output in the ketamine anaesthetized animals. However, it should be noted that a ketamine dose of 80 mg/kg i.p., in conjunction with coadministered benzodiazepine, is the recommended dosage regimen involving ketamine anaesthesia in the rat [15, 16].

Carotid catheterization is an integral part of the microsphere technique, and indeed many laboratory procedures. The insertion of a left ventricular catheter, via the right carotid artery, may be expected to alter blood flow to the brain. However, it has been demonstrated [17] that unilateral or bilateral carotid cannulation, in the rat, does not significantly affect cerebral blood flow, suggesting adequate blood flow must exist through collateral blood vessels. Contrary to this it has been shown, in the rat, that the presence of a right carotid artery catheter significantly reduced blood flow to the right hemisphere of the brain [18], indicating that carotid cannulation can affect cerebral blood flow.

In conclusion, all the anaesthetic regimens investigated in this study resulted in brain blood flows that were reduced compared to those previously reported in the conscious chronically catheterized rat [7, 8]. The differential influence of the anaesthetic regimens upon brain blood flow was

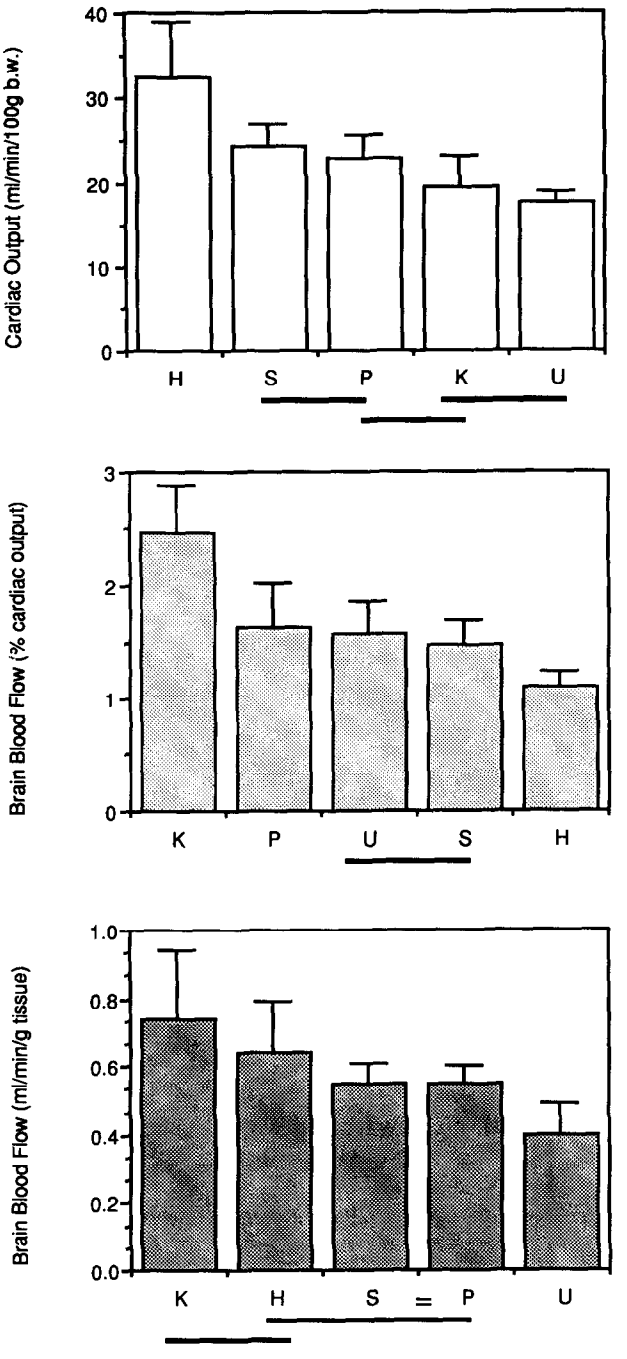

Fig. 1. Effects of anaesthetic regimens on mean cardiac output (ml/min/100 g body weight), mean % cardiac output distributed to brain and mean brain blood flow values (ml/min/g tissue) in rats (N = 6). Anaesthetic groups are ranked from left to right in descending magnitude of effect. Statistical comparisons were made using analysis of variance and Duncan's multiple range test and groups jointly underlined were not significantly different ($P > 0.05$) from each other.

maximal between ketamine/"Hypnovel" and urethane, whilst "Hypnorm/Hypnovel", "Saffan" and pentobarbitone anaesthesia gave brain blood flow values of intermediate magnitude. This differential influence of anaesthetic regimens upon brain blood flow is likely to be of significance to a number of neuropharmacological, biochemical or drug disposition based studies. The choice of laboratory anaesthetic employed should therefore form an important and integral part of the experimental design.

Acknowledgements—The authors wish to acknowledge the help of Dr C. R. Hiley and Dr M. R. Maclean, Department of Pharmacology, University of Cambridge, in setting up the experimental methodology.

Division of Pharmacology
The Welsh School of Pharmacy
University of Wales College of Cardiff
PO Box 13, Cardiff CF1 3XF U.K.

MARK GUMBLETON
GLYN TAYLOR
ROBERT D. E. SEWELL*
PAUL J. NICHOLLS

REFERENCES

1. McGrath JC and McKenzie JE, The effects of intravenous anaesthetics on the cardiovascular system of the rabbit. *Br J Pharmacol* **61**: 199–212, 1977.
2. Maekawa T, Conncezione T, Shapiro HM, Keifer-Goodman J and Kohlenberger RW, Local cerebral blood flow and glucose utilisation during isoflurane anaesthesia in the rat. *Anesthesiology* **65**: 144–151, 1986.
3. McDevitt DG and Nies AS, Simultaneous measurement of cardiac output and its distribution with microspheres in the rat. *Cardiovasc Res* **10**: 494–498, 1976.
4. Bell GJ, Hiley CR and Yates MS, The effects of four general anaesthetic agents on the regional distribution of cardiac output in the rat. *Br J Pharmacol* **61**: 126P, 1977.
5. Nies AS, Wilkinson GR, Rush BD, Strother JT and McDevitt DG, Effects on alteration of hepatic microsomal enzyme activity on liver blood flow in the rat. *Biochem Pharmacol* **25**: 1991–1993, 1976.
6. Nichols AJ and Hiley CR, Lack of effect of several barbiturates on liver blood flow. *Biochem Pharmacol* **34**: 3776–3778, 1985.
7. Idvall J, Aronsen KF, Nilsson L, and Nosslin B, Ketamine infusions, pharmacokinetics and clinical effects. *Br J Anaesth* **51**: 1167, 1979.
8. Idvall J, Influence of ketamine anaesthesia on cardiac output and tissue perfusion in rats subjected to hemorrhage. *Anesthesiology* **55**: 297–304, 1981.
9. Dawson B, Michenfedler JD and Theye RA, Effects of ketamine on canine cerebral blood flow and metabolism: modification by prior administration of thiopental. *Anesth Analg Curr Res* **50**: 443, 1971.
10. Takeshita H, Okuda Y and Sari A, The effects of ketamine on cerebral circulation and metabolism in man. *Anesthesiology* **36**: 69, 1972.
11. Haskins SC, Farver TB and Patz JD, Ketamine in dogs. *Am J Vet Res* **46**: 1855–1860, 1985.
12. Chen TS, Yeh FC, Chou YP and Chen HI, Effects of ketamine on circulatory functions and body tissue oxygenation in dogs under normal and hypovolemic conditions. *Proc Natn Sci Counc Repub China* **8**: 168–176, 1984.
13. Diaz FA, Bianco JA, Bello A, Beer N, Velarde H, Izquierdo JP and Jaen R, Effects of ketamine on canine cardiovascular function. *Br J Anaesth* **48**: 941–946, 1976.
14. Haskins SC, Farver TB and Patz JD, Cardiovascular changes in dogs given diazepam and diazepam-ketamine. *Am J Vet Res* **47**: 795–798, 1986.
15. Green CJ (Ed.), *Animal Anaesthesia*. Laboratory Animal Handbook No. 8. Laboratory Animals Ltd., London, 1979.
16. Green CJ, Knight J, Precious S and Simpskin S, Ketamine alone and combined with diazepam or xylazine in laboratory animals: a 10 year experience. *Laboratory Animals* **15**: 163–170, 1981.
17. Malik AB, Kaplan JE and Sabal TM, Reference sample method for cardiac output and regional blood determinations in the rat. *J Appl Physiol* **40**: 472–475, 1976.
18. Seyde WC and Longnecker DE, Anaesthetic influences on regional haemodynamics in normal and hemorrhaged rats. *Anesthesiology* **61**: 686–698, 1984.

* To whom correspondence should be addressed.

Biochemical Pharmacology, Vol. 38, No. 16, pp. 2748–2751, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

DNA-methylation in HL-60 cells treated with 3-deaza-(±)-aristeromycin and 3-deazaadenosine

(*Received* 5 *December* 1988; *accepted* 24 *February* 1989)

5-mCyt* occurs as a minor, modified base in the DNA of eukaryotes. Methylation of Cyt in DNA may be important in controlling gene expression, and alterations in 5-mCyt levels have been found in the DNA of carcinogen treated tissues and transformed cells (for reviews on the subject, see Refs. 1–4). Several reports indicate that 5-mCyt may be involved in the control of cellular differentiation [5–8].

The adenosine analogues c^3Ado and c^3Ari share the capacity to induce leukemia cell differentiation, inhibit AdoHcy hydrolase (EC 3.3.1.1), and perturb levels of transmethylation reactions in cells [9–11]. Hence the perturbation of transmethylation could be related to hypomethylation of DNA and alteration of gene expression in leukemic cells induced to functional maturation by the analogues [9, 10]. The level of DNA methylation in cells induced to differentiation by these compounds is therefore of interest. c^3Ado has multiple cellular targets [9, 12–15], and cytostasis and toxicity occur at concentrations lower than those needed for perturbation of transmethylations [9]. In order to study effects of the adenosine analogues on viable cells, the present investigation was undertaken using a dose schedule that caused cell cycle perturbation, cytostasis and differentiation but no overt toxicity [10, 11, 16, 17].

Materials and methods

Cells. HL-60 cells (a promyelocytic cell line) were grown in RPMI 1640 medium (Gibco, Paisley, U.K.) supplemented with 10% horse serum and 100 units of streptomycin/penicillin per ml, in an atmosphere of 5% CO_2 and 95% air. Cell counts were determined by the use of a hemocytometer chamber. Cell viability was assessed by exclusion of Trypan Blue.

* Abbreviations: c^3Ado, 3-deazaadenosine; c^3Ari, 3-deaza-(±)-aristeromycin; AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine; Adn, adenine; Cyt, cytosine; 5-mCyt, 5-methylcytosine, Thym, thymine; Gua, guanine; TFA, trifluoroacetic acid.