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DISTRIBUTION OF PENTAZOCINE IN BLOOD AND BRAIN OF THE BABOON FOLLOWING INTRAVENOUS INJECTION

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1 In the baboon the blood levels of pentazocine between 1 and 60 min after intravenous injection of 0.5 mg/kg were measured by a gas chromatographic technique. From cerebral arteriovenous differences it was shown that the peak of the brain concentration occurred within 15 min and probably within 10 min of intravenous injection. At the time of peak concentration about 10% of the injected dose was in the brain, while the corresponding value at 60 min was 2%.

2 The concentration of pentazocine in the brain was an order of magnitude greater than the concentration in cerebral venous blood both at 5 min and 60 min after injection. No major brain interregional differences were demonstrated. Cerebrospinal fluid from the cisterna magna did not yield values from which the cerebral concentration of pentazocine could be predicted.

Introduction

Pentazocine has been in routine clinical use since 1967 and a considerable amount of information has accumulated on its distribution, metabolism and therapeutic value. Little research has been carried out, however, on its concentration in the brain after intravenous injection of a dose in the clinical analgesic range. Furthermore, until recent years, analytical methods have been non-specific. It was therefore decided to study the brain uptake, removal and distribution of the drug, by gas liquid chromatographic analysis of blood and brain samples. Since this information is difficult to obtain in man, a non-human primate, the baboon, was the subject of the study.

Methods

Experimental procedure

Eight baboons (6.3-8.0 kg) were premedicated with phencyclidine hydrochloride (0.8-1.5 mg/kg) and anaesthesia was induced and maintained with halothane, nitrous oxide and oxygen. An endotracheal tube was inserted after intramuscular injection of suxamethonium chloride (50 mg) and the animals were artificially ventilated to normocapnia by a Palmer pump. Arterial blood gases and pH were measured (Radiometer) frequently. The concentration of inspired oxygen was adjusted so that PaO_2 remained above 100 mmHg. Muscle relaxation was maintained by injection of gallamine triethiodide (40 mg), suxamethonium chloride (50 mg) or pancuronium bromide (1 mg) intramuscularly at half-hourly intervals. The animal was kept at 37°C with heating lamps controlled by the oesophageal temperature.

The femoral artery was cannulated for blood pressure and blood gas measurements, as was the cisterna magna (except in one animal) for measuring pressure in the posterior fossa and for sampling cerebrospinal fluid (CSF). In two animals intracranial pressure measurements were also obtained by the method of Coroneos, McDowall, Gibson, Pickerodt & Keaney (1973). All pressures were measured electronically (Statham P23AA for blood pressure; Bell and Howell L221 for intracranial pressure) and written out on a multichannel recorder (Devices). Catheters were placed in the sagittal sinus and the jugular bulb for sampling cerebral venous blood. Pentazocine was injected into the inferior vena cava through a catheter inserted via a femoral vein. Cerebral blood flow was measured by the xenon clearance technique in four animals.

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When the surgical preparation was complete the inspired halothane concentration was reduced to 0.5 v/v% and 1 h later control measurements were made. After a blood sample had been taken from the femoral artery, pentazocine (0.5 mg/kg) was injected and arterial and cerebral venous blood was sampled at 1, 2, 3, 4, 5, 10, 15, 30, 45 and 60 minutes. CSF was obtained in six experiments between 5 and 60 min after injection. Six animals were killed at 60 min and two at 5 min after injection. The brain was removed and dissected into five regions, cortical grey matter, white matter, thalamus, brain stem and cerebellum; each sample was weighed. Brain and blood samples were stored at -4°C.

Estimation of pentazocine in blood

Pentazocine was assayed by a modification of the gas-liquid chromatographic method of Beckett, Taylor & Kourounakis (1970). The distribution of pentazocine between plasma and red blood corpuscles (RBC) was approximately 1 : 1. In these experiments, all measurements were made on whole blood. Blood (1-5 ml) was mixed with 1 ml α-methadol solution (2.5 μg/ml, base equivalent) as internal standard and 1.5 ml 20% w/v NH_4Cl-ammonia buffer, pH 9.4. The blood was extracted three times with 15 ml freshly distilled ether and the ether layers pooled. The ether fraction was extracted twice with 10 ml 0.1 M HCl and the acid extracts washed with 5 ml ether, which was discarded. After the addition of 5 ml ammonia buffer, the aqueous phase was extracted twice with 5 ml ether. The pooled ether extracts were transferred to a capillary-bottomed tube and evaporated to approximately 500 μl under a stream of nitrogen gas. *n*-Butanol (10 μl) was added and the remaining ether evaporated at 65°C under N_2. The butanol residue, up to 4 μl, was injected on to the gas chromatographic column.

Estimation of pentazocine in brain tissue

Brain tissue (1-5 g) was homogenized in an equal volume of 0.1 M HCl. Samples were transferred to a previously weighed tube, and the tissue weight determined. The homogenate was extracted four times with 30 ml petroleum ether (b.p. 40°-60°C) and the organic phase discarded. α-Methadol solution (1 ml) was added, followed by 5 ml ammonia buffer. The suspension was extracted with 35 ml benzene with mechanical shaking and centrifugation. The benzene layer was transferred quantitatively to a second tube and extracted with 15 ml 0.1 M HCl. The acid layer was washed with 5 ml ether, 5 ml ammonia buffer added, and extracted twice with 5 ml ether. The pooled ether extracts

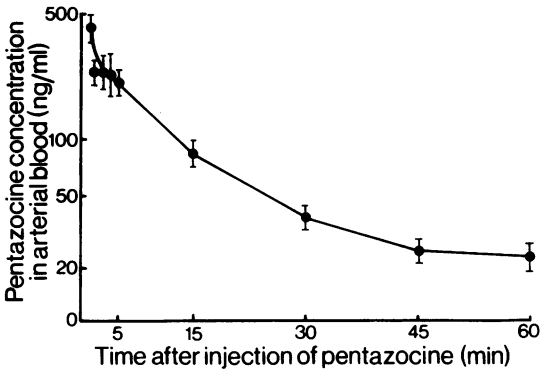

Fig. 1 Pentazocine concentrations in arterial blood after injection of 0.5 mg/kg into the inferior vena cava. The points are means of five experiments (except at 15, 45 and 60 min, $n = 6$); the vertical bars are the s.e. of the means.

were transferred to a capillary-bottomed tube, evaporated to 500 μl under a stream of nitrogen gas. *n*-Butanol (10 μl) was added, and the remaining ether evaporated at 65°C under N_2. (Benzene rather than ether was used in the initial stages to prevent flocculation.)

Gas chromatographic conditions

A Perkin-Elmer F-11 linked to a Hitachi Model 159 pen recorder, 2 mV full scale deflection. Inlet temperature 265°C, column temperature 250°C, column packing 4% Apiezon L on Chromasorb W AW-DMCS 80-100 mesh, 1 m x 6 mm borosilicate glass. Carrier gas O_2-free nitrogen flowing at 55 ml/minute. Detector, flame ionization with hydrogen at 140 kN/m^2 and compressed air at 170 kN/m^2. Attenuation, 1×10^2 for internal standard, 10 or 20 for pentazocine. Retention times: 1-methadol, 7 min; pentazocine, 12 minutes.

Specificity and recovery

No endogenous peaks were noted at either the pentazocine or α-methadol retention times in pentazocine-free blood or brain tissue. Recovery ranged from 85-95% from blood, and 65-82% from brain, with averages 92% and 76% respectively.

Calculation of results

Assay values were calculated by reference to appropriate calibration curves. These were constructed by adding known amounts of pentazocine

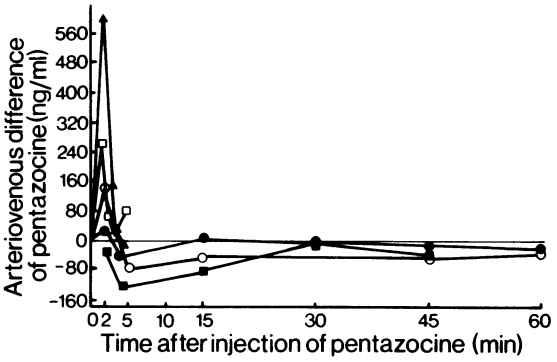

Fig. 2 Differences in pentazocine concentrations in blood from femoral artery and sagittal sinus after injection of 0.5 mg/kg into the inferior vena cava. On the ordinate scale, + indicates higher, and − lower value in arterial blood. (●) Experiment 4; (○), 5; (■), 6; (□), 7; (▲), 8.

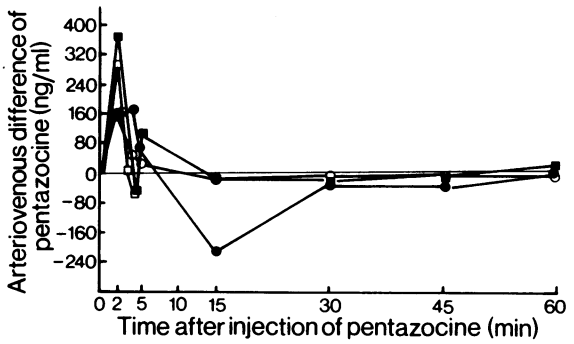

Fig. 3 Differences in pentazocine concentration in blood from femoral artery and jugular bulb after injection of 0.5 mg/kg into the inferior vena cava. On the ordinate scale, + indicates higher, and − lower value in arterial blood. (●) Experiment 1; (○) 2; (■) 3; (□) 4.

to blood (range, 0-250 ng/ml) and brain homogenate (range, 0-2.5 μg/g) and by proceeding as detailed above. The calibration curves were linear over the ranges studied. The peak areas of α-methadol and pentazocine were measured by triangulation, and the pentazocine α-methadol peak ratio was plotted against authentic pentazocine concentration. Experimental pentazocine values were determined by direct inspection.

Results

The levels of pentazocine in arterial blood are shown in Fig. 1; there was a rapid decline from the initial peak for 15 min followed by a slower rate of decay. In experiments 2, 3, 4 and 6, a line fitted by least squares for the concentrations between 15 and 60 min gave half-life values of 40, 52, 20 and 53 minutes. Second peaks occurred in animals 1 and 5. The time course of the differences in the pentazocine concentration between arterial blood and cerebral venous blood in the sagittal sinus or in the jugular bulb are given in Figs. 2 and 3 respectively. The phase of brain uptake, as indicated by positive arteriovenous differences, was complete by 15 min and indeed by 10 min in the three animals in which samples were obtained at this time (not shown).

There were no consistent differences between the pentazocine concentrations in different areas of the brain (Table 1) but considerable differences in mean brain concentration existed between animals. That these latter differences were not at random, is demonstrated by the relative consistency of the brain/cerebral venous blood ratios for pentazocine which ranged only between 8 and 14. At 60 min after injection, the brain/arterial blood ratios were higher but at 5 min the brain/arterial blood ratios were similar to the brain/cerebral venous blood ratios because brain and blood were closest to equilibrium around this time (Figures 2 and 3).

Pentazocine concentrations in CSF from cisterna magna were always considerably lower than those in cerebral venous blood (Table 2) and varied in an apparently random manner in relation to blood levels.

Injection of pentazocine into the inferior vena cava produced no change in blood pressure within the first minute. Thereafter, blood pressure decreased but this fall was probably due to the arterial and cerebral venous blood sampling which was proceeding after the first minute. The pressure in the CSF rose during the first minute by 2.5 ± 0.6 mmHg. The rapid injection of fluid into the inferior vena cava may have been responsible; however, such effects normally subside within 10-15 s whereas the increase following pentazocine injection persisted for 50 seconds. Cerebral blood flow did not change significantly during the 60 min following injection of pentazocine.

Discussion

The pattern of metabolism of pentazocine is similar in non-human primates and man (Archer, Pierson, Pittman & Aceto, 1972). However, its half-life in man is difficult to ascertain from the literature. Using a fluorometric method of analysis

Table 1 Pentazocine concentrations (ng/g) in various regions of the brain

	Expt no.	Cortical grey matter	Sub-cortical white matter	Thalamus	Brain stem	Cerebellum	Brain average	Ratio of brain /arterial blood	Ratio of brain /cerebral venous blood
5 min after injection	7	3100	2700	4200	2600	4400	3400	10	13**
	8	2100	1500	1800	1700	1600	1740	8	8**
60 min after injection	1	410	300	–	370	260	335	28	–
	2	160	210	210	280	260	224	17	9*
	3	560	210	400	450	500	424	12	14*
	4	298	979	683	383	100	489	28	12**
	5	853	774	1095	884	648	851	23	14**
	6	720	876	1061	601	360	724	32	–

Cerebral venous blood was obtained either from the jugular bulb (*) or sagittal sinus (**).
Pentazocine was injected intravenously.

of pentazocine, Berkowitz, Asline, Schnider & Way (1969) found in man that, between 15 and 120 min after intravenous injection, plasma levels declined at a constant rate of decay with a half-life of 111 minutes. After intramuscular administration the half-life was 126 minutes. In making these calculations these workers assumed a two compartmental pharmacokinetic model. This approach is not completely satisfactory but is useful in assessing drugs which have a therapeutic effect correlated with plasma distribution kinetics. That pentazocine is such a drug is apparent from Figs. 3 and 4 of the above-quoted paper. Replotting the data of Davison and Scrime (Sterling-Winthrop Research Institute, personal communication), which are based on measurements in volunteers who received 30 mg of pentazocine intravenously, we obtained half-life values of 71, 54, 47, 60 and 57 min; similar treatment of the data of Beckett *et al.* (1970) yielded values of 39, 33, 50 and 33 minutes. Our results, in the baboon, were similar to the mean half-life values obtained by these last two groups of workers who also used a gas liquid chromatographic method for the analysis of pentazocine.

Burt & Beckett (1971) and Berkowitz *et al.* (1969) found that second peaks in the plasma concentration curves occur within 1 h of intravenous injection; these second peaks have been attributed to enterohepatic recycling by the former workers. Similar second peaks occurred in two of the present experiments.

The brain reaches distribution equilibrium with pentazocine in the blood within the first 15 min and probably within 10 minutes. Thereafter brain concentration continuously falls from the peak value reached within 10 min after injection. At 15 min, the arterial blood concentration of pentazocine in the baboon was 83 ± 14 ng/ml while in man, under similar conditions, it was 82 ±9 ng/ml (Davison & Scrime, personal communication). It is likely, therefore, that a similar time pattern of brain uptake and peak brain concentration occurs in man and, in conformity with this view, Berkowitz *et al.* (1969) have shown that peak analgesia is attained within 15 min of intravenous injection.

The primary objectives of this study were to determine the brain/blood ratio for pentazocine and to test whether brain pentazocine levels could be assessed from analysis of CSF. Brain concentrations would be expected to be more closely related to the concentrations in cerebral venous blood than in arterial blood. Sixty minutes after intravenous injection, the brain concentration was 12 times higher than that in cerebral venous blood and 22 times higher than that in arterial blood. Five minutes after injection, the brain concentration was higher than at 60 min and the concen-

Table 2 Pentazocine concentrations (ng/ml) in CSF and cerebral venous blood

Expt no.	Interval after injection (min) 5-6 CVB	5-6 CSF	10 CVB	10 CSF	15 CVB	15 CSF	30 CVB	30 CSF	45 CVB	45 CSF
1*	220	10	–	–	336	10	64	48	56	10
3*	131	5	–	–	128	71	–	–	–	–
4**	110	0	71	41	–	–	–	–	33	0
5**	204	59	–	–	123	66	106	67	–	–
6**	153	50	127	0	123	0	39	0	–	–
7**	243	67	–	–	–	–	–	–	–	–

CSF was obtained from the cisterna magna and cerebral venous blood (CVB) from the jugular bulb (*) or the sagittal sinus (**). No pentazocine was found in CSF at 60 min (Expt 1 and 3).

tration ratios, brain to cerebral venous blood and brain to arterial blood, were both about 10. Pittman & Erdmansky, quoted by Archer *et al.* (1972), found in monkeys that the concentration of pentazocine in the brain, 60 min after intramuscular injection, was 10 times higher than in arterial blood.

Pentazocine concentrations in the CSF were lower than in cerebral venous blood and there was no constant relationship between the two sets of values. For an assessment of the concentration of pentazocine in the brain of man, cerebral venous blood levels would be more reliable indicators than the CSF levels; approximate quantitative estimates could be obtained on the basis of the brain/cerebral venous blood ratio of 12 found in the present experiments. A level of 40 ng/ml of pentazocine in peripheral venous blood was found by Berkowitz *et al.* (1969) to produce moderate analgesia in man, but there are no equivalent human data available with measurements of pentazocine in cerebral venous blood.

The absence of differences in the regional distribution of pentazocine in the brain agrees with the results of Berkowitz & Way (1971) and yields no information on site of drug action.

After intravenous injection of pentazocine in the baboon there was no significant change in blood pressure although an increase has been reported in patients (Scott & Adgey, 1972). An elevation of CSF pressure in the first minute after injection may have been due to a rise in central venous pressure, which has been described in man by Brown (1972), or to an initial vasodilatation of cerebral vessels. The xenon clearance technique of cerebral blood flow measurement cannot be used to detect such transient changes. No difference from the control flow was seen during the 60 min after giving racemic pentazocine. We cannot confirm, therefore, the suggestion of Berkowitz & Way (1971) that pentazocine may decrease cerebral blood flow.

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