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Morphine concentrations in serum, brain and cerebrospinal fluid in the rat after intravenous administration of a single dose

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Morphine has been determined in serum, cerebrospinal fluid (c.s.f.) and in five brain regions in the rat after a single intravenous dose, using high performance liquid chromatography with an electrochemical detector. In pure solution 50 pg morphine and 200 pg naloxone could be detected. Maximal concentrations of morphine were observed in serum and in most brain regions 5 min after administration of morphine. There was a rapid decline in morphine concentrations during the first 30 min, in serum and in all brain regions studied. The morphine concentration in the c.s.f. was constant for the first 30 min, but 30 min later there was a dramatic increase, suggesting that elimination through the c.s.f. could be an important way of eliminating morphine in the central nervous system.

The major effects of morphine and other opiates are generally considered to be of central origin. Because of its low concentration in the central nervous system (c.n.s.), there have been methodological difficulties in studying its disposition in those tissues (Mulé 1971). Recently the disposition of morphine in subcortical rat brain regions was studied after a single intravenous injection and at three different doses (Plomp et al 1981). In that study morphine was measured using methods such as radioimmunoassay and gas-liquid chromatography. It has also been shown that morphine can be analysed in solution and in blood using high-performance liquid chromatography (h.p.l.c.) combined with electrochemical detection (White 1979; Peterson et al 1980; Wallace et al 1980) and recently also in specific brain regions (Raffa et al 1982). In the present work we have examined the disposition of free morphine in serum, various brain regions and cerebrospinal fluid (c.s.f.) in the rat. Morphine was administered as a single intravenous dose and analysed using h.p.l.c. with an electrochemical detector. To our knowledge this is the first study in the rat, where morphine concentrations have been followed in serum, brain and in the c.s.f. simultaneously.

MATERIALS AND METHODS

Animals. Male or female Sprague-Dawley rats (Anticimex, Sollentuna, Sweden), 175–275 and 135–160 g respectively, were fed the Ewos-Anticimex commercial type pelleted diet, R3 with free access to water.

Drugs. Morphine HCl (Pharmacopea Nordica) and pentobarbitone (60 mg ml^{-1}) were obtained from ACO, Solna, Sweden. Naloxone HCl was a generous gift from Endo Laboratories Inc., Garden City, N.Y.

Method

Morphine chloride 10 mg kg^{-1} was administered intravenously as a solution 5 mg ml^{-1} in 0.9% NaCl (saline). At different times after administration the rats were placed in a stereotaxic frame and under light anaethesia with pentobarbitone, 30 mg kg^{-1} i.p., the atlanto-occipital joint was exposed, and a 23 gauge needle inserted for collection of c.s.f. Immediately after its collection the rats were decapitated and truncal blood collected. Both c.s.f. and blood were centrifuged. The brains were rapidly removed, blotted with filter paper and placed on ice-cold glass dishes and dissected under a microscope into cortex, striatum, midbrain, cerebellum and spinal medulla. (Spinal medulla consisted of the lower cervical and upper thoracic regions.) Serum, c.s.f. and the sampled brain regions were frozen (−80 °C) until analysis.

Extraction of morphine

To the sample in a small conical plastic tube was added 0·5 ml 0·1 M $HClO_4$ and 10 µl of a solution of naloxone 10 µg ml^{-1} as an internal standard. After

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sonication (Branson cell disruptor, B15) and centrifugation, the supernatant was transferred to a test tube and 0·5 ml 1 M carbonate buffer, pH 9, and 2 ml $CHCl_3$ were added. After extraction with shaking and centrifugation, 1·8 ml of the organic layer was transferred to a new test tube and evaporated under a stream of filtered nitrogen. The residue was taken up in 200 µl methanol. 10 or 20 µl of the methanol solution was injected into the h.p.l.c. system.

H.p.l.c. separation and amperometric determination of morphine

We used the solvent delivery system M-45 and U 6 K loop injector (Waters Assoc., Milford, Mass., USA) equipped with the electronic controller LC-4 and a glassy carbon electrode TL 5A (Bioanalytical Systems, LaFayette, IN, USA). The column was a Nucleosil C_{18} analytical column with 5 µm particles and dimensions 200 × 4·6 mm coupled in series with a Nucleosil C_{18} precolumn with 10 µm particles. The column had 2500 theoretical plates m^{-1} for morphine. The mobile phase consisting of sodium phosphate buffer pH 5.6–ethanol (90:10 v/v) was degassed and filtered (Milipore 0·2 µ) and delivered at ambient room temperature at a rate of 1·0 ml min^{-1}. The back pressure was approximately 1500 p.s.i. at this flow rate. A current-voltage curve for morphine was in close agreement with that obtained by White (1979). We also found it most favourable to choose a potential of 0·6 to 0·8 V across the electrochemical detector, where the current-voltage curve had a plateau. The detector response could be increased by increasing the electrode potential. However, an increased detector potential resulted in a greater noise level and also in a decreased specificity of the detector. The electrochemical detector could be used for about twenty determinations of morphine in tissue extracts or body fluids before its function was disturbed by any contamination. In most cases the performance of a contaminated electrode could be restored by stopping the determinations and only deliver the mobile phase through the chromatographic system. Amounts of 50 pg morphine could be detected corresponding to a signal-to-noise ratio 3·3. The minimal detectable amount of naloxone was about 200 pg.

The peak height ratios of morphine to internal standard, naloxone on regression analysis of 0·05–1·20 ng showed a coefficient of correlation 0·999, a slope 0·652 and a y-intercept 0·030.

Table 1 shows the precision of the method at spiked morphine levels for each biological matrix. Recovery of 500 ng morphine or 500 ng naloxone to brain tissue was 67 ± 7·3% (mean ± s.d., n=6) and 107 ±6·6% (mean ± s.d., n=6) respectively. The corresponding values for 500 ng morphine or 500 ng naloxone added to serum were 71 ± 5·7% (mean ± s.d., n=6) and 99 ± 5·3% (mean ± s.d., n=6). In c.s.f. the recovery of 500 ng morphine was 71 ± 3·8% (mean ± s.d., n=6) and of 500 ng naloxone 105 ± 13·3% (mean ± s.d., n=6). All morphine values in brain tissue, serum or c.s.f. were corrected for recovery.

Table 1. Precision of the extraction method with 500 ng morphine added to brain tissue, serum or c.s.f. (n=6).

Tissue/ body fluid	Wet wt or vol	Calc (ng)	s.d. (ng)
Brain	34–54 mg	333	36
Serum	0·3 ml	354	29
C.s.f.	0·1 ml	353	47

Statistical analysis. The two-tailed Student's *t*-test was used. A *P*-value of less than 0·05 was considered significant.

RESULTS

Fig. 1 shows an example of chromatograms of morphine in pure solution and in one brain region. Similar chromatograms were obtained with extracts from serum and c.s.f.

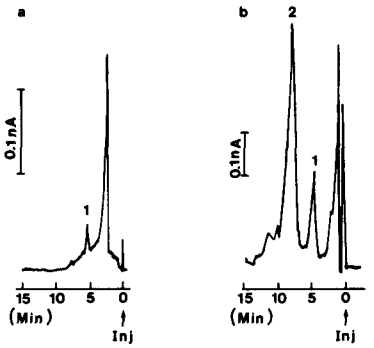

FIG. 1. Chromatograms showing (a) morphine 50 pg in standard solution, (b) morphine in the striatum of a rat given morphine 10 mg kg^{-1} i.v. 60 min before death and with naloxone added as internal standard. Peak 1, morphine and 2, naloxone.

Morphine in serum

The morphine concentration in serum reached a maximum 5 min after its administration (Table 2). Thereafter the concentration rapidly fell within 30 min. After 60 min the concentration did not significantly differ from that after 30 min.

Table 2. Morphine concentrations in serum, c.s.f. (ng ml^{-1}) and in different brain regions (ng g^{-1}) various times after intravenous administration of morphine HCl 10 mg kg^{-1}. Each value represents the mean ± s.e., (n).

	Time (min) after i.v. administration of morphine			
	a 5	b 15	c 30	d 60
Tissue or body fluid				
Serum	3859±1012 (6)	468±39 (5)	146± 9 (5)	177± 57 (4)
C.s.f.	279± 34 (4)	256±61 (4)	279±34 (5)	1489±566 (3)
Cortex	160± 26 (6)	227±24 (5)	60± 4 (5)	63± 10 (4)
Striatum	209± 36 (6)	218±26 (5)	–	30± 6 (4)
Midbrain	204± 17 (5)	137±19 (5)	–	49± 9 (3)
Cerebellum	303± 37 (6)	282±30 (5)	107±11 (5)	93± 29 (4)
Spinal medulla	269± 30 (6)	219±19 (5)	100±10 (5)	154± 49 (4)

Morphine in c.s.f.

Morphine was present in the c.s.f. 5 min after administration. The concentration did not significantly change during the first 30 min. However, a dramatic increase was observed 30 min later.

Morphine in brain

In agreement with earlier findings in subcortical brain areas (Plomp et al 1981), we found that in most of the brain regions examined the morphine concentration reached a maximum 5 min after injection. However, in the cortex the highest concentration was after 15 min. Irrespective of time, the highest concentrations were observed in cerebellum and spinal medulla, although significant differences in the regional concentration were frequent only 30 min after morphine administration. Because of technical problems the concentrations after 30 min were not measured in the striatum and in the midbrain. However, in the other regions there was a rapid decline between 15 and 30 min. No further decrease in concentration was observed between 30 and 60 min. At no time did the concentrations in the brain regions exceed corresponding serum values. The concentrations of morphine in cerebellum and spinal medulla were similar to those found in c.s.f. 5 and 15 min after drug administration. In other regions the concentrations were lower than in the c.s.f.

DISCUSSION

In agreement with others we found a maximal concentration of morphine in serum 5 min after its intravenous administration (Dahlström & Paalzow 1978; Plomp et al 1981) and also a rapid decrease during the first 60 min. A rapid decline was also observed in the brain. This finding is similar to the results obtained by Dahlström & Paalzow (1978) but differs from those of Plomp et al (1981). There were regional differences in concentration. In previous studies higher concentrations have been found in subcortical areas rather than in other brain regions

Table 3. Comparisons between various treatments. *P*-values.

	Time (min) after i.v. administration of morphine			
	a 5	b 15	c 30	d 60
Tissue or body fluid				
A Serum	—	Aa–Ab <0·02 Ab–Ac <0·001 Ab–Ad <0·005	Aa–Ac <0·01	Aa–Ad <0·02
B C.s.f.	Aa–Ba <0·02	Bb–Bd <0·05 Ab–Bb <0·02	Bc–Bd <0·025 Ac–Bc <0·01	Ba–Bd <0·05 Ad–Bd <0·05
C Cortex	Aa–Ca <0·005 Ba–Ca <0·02	Ab–Cb <0.001	Ca–Cc <0·01	Ca–Cd <0·025
D Striatum	Aa–Da <0·005	Ab–Db <0·001	—	Da–Dd <0·005 Ad–Dd <0·05 Bd–Dd <0·05 Cd–Dd <0·05
E Midbrain	Aa–Ea <0·01 Ba–Ea <0·05	Ea–Eb <0·05 Ab–Eb <0·001 Cb–Eb <0·025 Db–Eb <0·05	—	Ea–Ed <0·001 Bd–Ed <0·05
F Cerebellum	Aa–Fa <0·005 Ca–Fa <0·02	Ab–Fb <0·005 Eb–Fb <0·005	Fa–Fc <0·005 Ac–Fc <0·005 Bc–Fc <o·001 Cc–Fc <0·001	Fa–Fd <0·005 Bd–Fd <0·05
G Spinal medulla	Aa–Ga <0·005 Ca–Ga =0·025	Ab–Gb <0·001 Eb–Gb <0·02	Ga–Gc <0·005 Ac–Gc =0·005 Bc–Gc <0·001 Cc–Gc =0·01	Bd–Gd <0·05 Dd–Gd =0·05

(Mulé 1971). In the present study the highest concentrations were in cerebellum and spinal medulla. In opiate receptor-rich brain regions (Kuhar & Uhl 1979), such as midbrain and striatum, the concentrations were relatively low. Hence, the prevalence of opiate receptors does not seem to influence the concentration of morphine. In the c.s.f. the values were about as high as the highest concentrations in the brain and values were constant from 5–30 min. However, 60 min after administration there was a dramatic increase in c.s.f. morphine simultaneously with low brain concentrations. Thus, elimination via the c.s.f. could be a route for elimination of brain morphine. The late appearance of the high values in the c.s.f. suggests that the drug could be retained in the brain (Plomp et al 1981). Compartments outside the brain might also contribute to the high c.s.f.-values seen. In addition pentobarbitone anaesthesia may have influenced the transport of morphine both into and out of the c.s.f., although it does not seem likely that this effect would be maximal after about 60 min.

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