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DETERMINATION OF METHYLPREDNISOLONE IN CENTRAL NERVOUS TISSUE AND PLASMA USING NORMAL-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A sensitive and specific high-performance liquid chromatographic technique is described for the quantitative measurement of the synthetic glucocorticoid methylprednisolone in central nervous tissue (spinal cord) and plasma. Following intravenous administration, methylprednisolone is extracted from spinal cord tissue with diethyl ether—methylene chloride (60:40, v/v). The extract is washed sequentially with alkali, acid and water, concentrated, then chromatographed on an NH_2 column using a mobile phase of methylene chloride—isopropanol (85:15, v/v). Steroid elution is monitored with an ultraviolet detector set at 254 nm. Such a system has a detection limit of 2.8 ng methylprednisolone. Extraction of methylprednisolone from spinal cord tissue is linear with tissue concentration and the recovery is around 70%. Endogenous hydrocortisone or other metabolites in the tissue do not interfere with the methylprednisolone peak. A description of the quantitation of methylprednisolone in cat lumbar spinal cord and plasma samples after single intravenous doses of methylprednisolone sodium succinate is given.

INTRODUCTION

Recent studies from this laboratory have demonstrated acute effects of the synthetic glucocorticoid methylprednisolone (MP) on certain neurophysiological [1, 2] and neurochemical [3—5] parameters of spinal cord function. In order to correlate the amount of MP at the site of action with these effects, a reliable method was needed for the extraction and determination of glucocorticoid concentrations in the spinal cord of treated animals.

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Methods have been presented for assaying MP in plasma samples utilizing both reversed-phase [6, 7] and normal-phase [8—10] high-performance liquid chromatography (HPLC). However, to adapt any of these methods to the use of tissue samples, an effective extraction procedure is required and the assay must be of sufficient sensitivity to detect the smaller amount of the drug that would likely be present in the tissue compared to plasma. In addition, the method must resolve MP and its internal standard from all the other steroidal compounds endogenously present in the central nervous system (CNS). In this report we describe a simple method using normal-phase HPLC with UV detection for the measurement of MP in both CNS tissue and plasma samples.

EXPERIMENTAL

Materials

Triamcinolone acetonide (TA) and hydrocortisone (HC) were obtained from Sigma (St. Louis, MO, U.S.A.). Methylprednisolone sodium succinate (Solu-Medrol) (MP) and methylprednisolone were gifts from Upjohn (Kalamazoo, MI, U.S.A.). The HPLC solvent, methylene chloride and isopropanol were purchased from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Other reagents were from Fisher (Cleveland, OH, U.S.A.) and were the highest grade available.

Instrumentation

A Varian (Walnut Creek, CA, U.S.A.) high-performance liquid chromatograph Model 5020 consisting of a gradient capacity pump, a Valco loop injector, Micropak NH_2 column (30 cm × 4 mm, 10 μm particle size), a fixed-wavelength (254 nm) ultraviolet detector, a CDS 111 integrator and a Model 9176 recorder were used in these studies. The mobile phase was an isocratic mixture of methylene chloride—isopropanol (85:15) pumped through the column at a flow-rate of 2 ml/min.

Animal preparation and administration of drugs

Adult mongrel cats of either sex weighing 1.8—4.0 kg were anesthetized with alpha chloralose from Sigma (80 mg/kg intravenously). A dorsal laminectomy was performed to expose the lumbar spinal cord from the 1st through the 4th lumbar levels. Methylprednisolone sodium succinate (Solu-Medrol) in a concentration of 60 mg/ml was injected into a brachial vein as a single dose per animal of either 15, 30, 60 or 90 mg/kg. Samples (1 ml) of carotid arterial blood were collected in heparinized syringes just prior to the removal of approximately a 1.5-cm cross-section of the lumbar spinal cord at one hour after MP administration. The section of spinal cord was immediately frozen in liquid nitrogen and stored at —70°C until assay. Blood samples were immediately centrifuged at 1000 *g* for 5 min and the plasma was transferred to clean tubes, frozen and stored at —70°C until assay.

Sample preparation

A 200—400 mg portion of frozen spinal cord was homogenized with a Polytron Homogenizer (Brinkman Instruments, Westbury, NY, U.S.A.) at 4°C

in 5 ml of diethyl ether—methylene chloride (60:40). The homogenate was incubated at room temperature for 15 min with shaking to facilitate extraction of the lipophilic MP from the highly lipid nervous tissue. The extracted homogenate was centrifuged at 700 *g* for 5 min and the resulting supernatant fraction was sequentially washed with 1-ml volumes of 0.1 *N* sodium hydroxide, 0.1 *N* hydrochloric acid and glass-distilled water. After each washing, the samples were centrifuged at 700 *g* for 5 min and the organic phase was transferred to a clean tube for the next washing. The final extract was transferred to a small centrifuge tube where it was evaporated to dryness under a stream of dry nitrogen. The residue was reconstituted in 0.3 ml of methylene chloride—isopropanol (85:15) containing 500 ng of TA as an internal standard, and a 100-μl aliquot was injected into the chromatographic system.

Plasma methylprednisolone was extracted by adding 0.5 ml plasma to 5 ml diethyl ether—methylene chloride (60:40). The sample was then extracted and prepared as described for the tissue samples, dried, dissolved in 500 μl of methylene chloride—isopropanol (85:15) containing 10 μg TA as an internal standard, and a 25-μl aliquot was injected into the chromatographic system.

Standard solutions of TA, HC and MP in isopropanol were prepared from stock solution of 0.4 mg/ml. MP was quantitated on the basis of the ratio of the peak area of MP to that of TA. The results were expressed as μg of MP per g wet tissue or per ml plasma.

RESULTS AND DISCUSSION

The general purpose of this study was to develop a technique which would allow a determination of glucocorticoid concentrations in central nervous tissue using HPLC with UV detection. In the present instance, the glucocorticoid in question is the synthetic MP and the tissue of interest is the spinal cord. In addition to the usual criteria for good chromatography, a suitable technique for this purpose must include: (1) the choice of a suitable internal standard with good separation from the glucocorticoid to be measured, (2) a clear separation of the measured glucocorticoid from other steroids such as endogenous HC (i.e., cortisol) which is concentrated in brain and other tissues [11], and (3) an extraction procedure capable of removing the lipophilic steroid from neuronal membranes and myelin. The method described in this report meets these criteria.

Fig. 1 shows a chromatogram of a mixture of 100 ng each of TA, HC and MP demonstrating the clear separation of these three glucocorticoids using the column and mobile phase described above. The longest retention time was approximately 6 min for MP, the least polar of the three. Furthermore, the detector response to MP was found to be linear over a ten-fold range of MP ($r = 0.99$).

Fig. 2 shows a typical chromatogram of a lumbar spinal cord sample from an untreated cat which had been spiked with 500 ng each of TA, HC and MP. Chromatograms of unspiked spinal cord extracts from untreated animals (not shown) showed no major endogenous peaks which would interfere with the resolution of the three glucocorticoids in question.

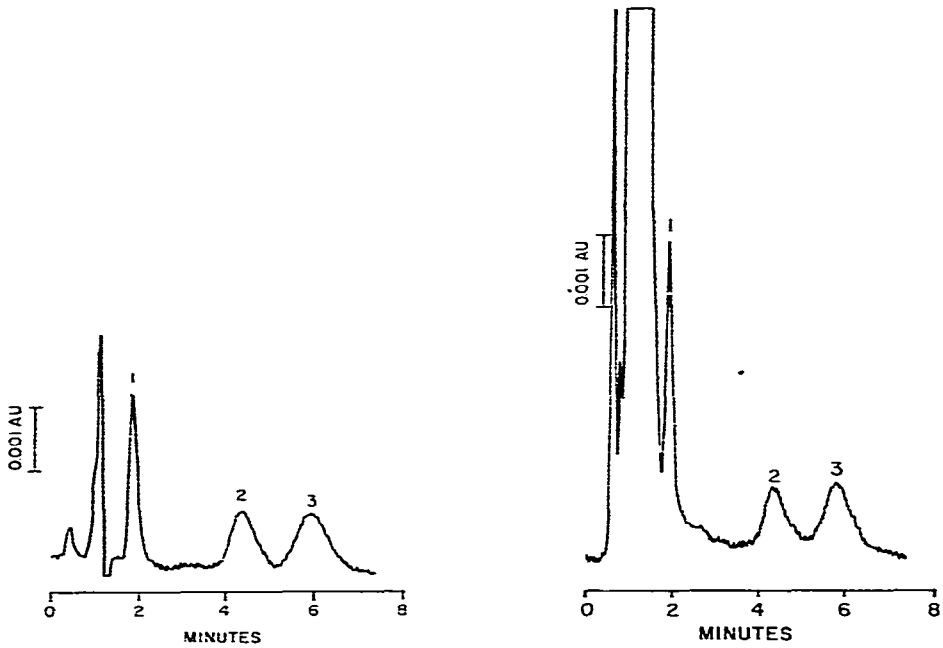

Fig. 1. High-performance liquid chromatogram showing separation of a mixture containing 100 ng each of TA = 1 (internal standard), HC = 2, and MP = 3. Injection volume was 100 μl.

Fig. 2. High-performance liquid chromatogram of a 200-mg cat lumbar spinal cord extract spiked with 500 ng each of TA (internal standard) = 1, HC = 2, and MP = 3. Injection volume was 100 μl.

Fig. 3 displays a chromatogram of a spinal cord extract from an animal injected intravenously with 90 mg/kg of the sodium succinate ester of MP 1 h prior to cord removal. There is no discernable HC peak because the amount endogenously present in the spinal cord was below the sensitivity required to measure the MP. For MP, the detection limit using this assay, determined as that quantity measured at twice the noise level, was 2.8 ng in a 100-μl injection volume or 8.4 ng in a 200-mg spinal cord sample. The intra-assay variability with triplicate determinations of a single sample was approximately 4%. The MP measured in the extract was determined to be the free non-esterified form since the retention time corresponded to that obtained for the MP standard. Fig. 4 shows a typical chromatogram of MP extracted from the plasma of a cat injected with 90 mg/kg of the sodium succinate ester of MP 1 h before sampling.

The extraction procedure with diethyl ether—methylene chloride (60:40) was arrived at by trial and error. Use of methylene chloride alone as described by others [6—10] for extraction of glucocorticoid from plasma samples was not effective in removing the highly lipophilic MP from the cord tissue samples. The effectiveness of the extraction described here is demonstrated in Fig. 5 which shows a linear relationship between cord sample size and MP content.

The recovery of MP from the tissue was estimated by adding 500 ng MP to a 200-mg spinal cord sample from an untreated cat either before or after the

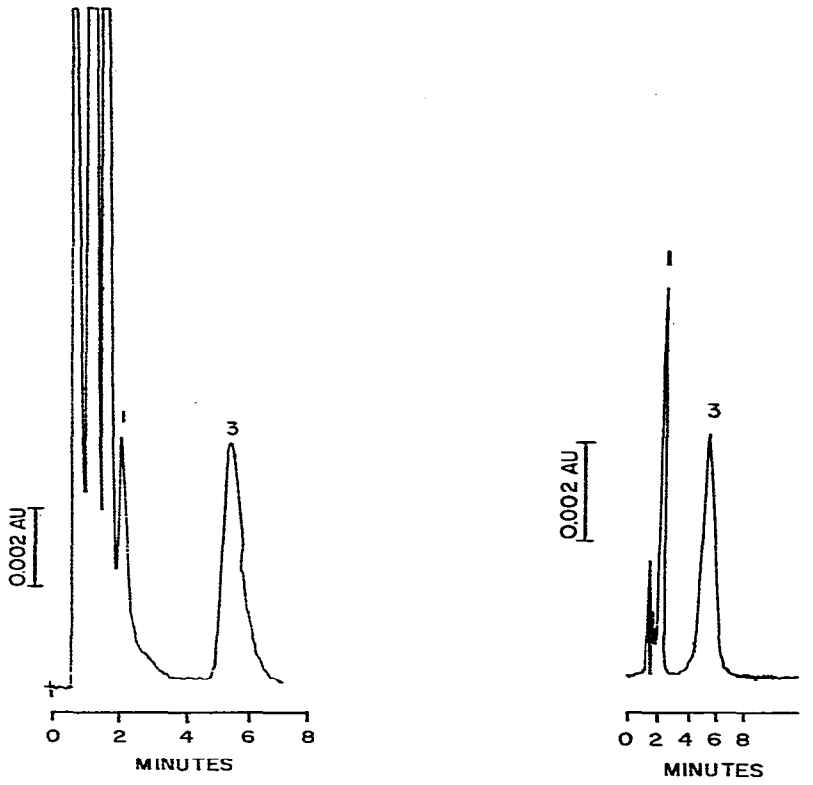

Fig. 3. High-performance liquid chromatogram of a lumbar spinal cord sample from a cat injected intravenously with 90 mg/kg MP sodium succinate at 1 h before cord removal. TA (internal standard) = 1, and MP = 3. Injection volume was 100 μl.

Fig. 4. High-performance liquid chromatogram of a plasma sample from a cat injected intravenously with 90 mg/kg MP sodium succinate at 1 h before sampling. TA (internal standard) = 1, and MP = 3. Injection volume was 25 μl.

tissue extraction procedure. Peak height ratios of MP to the TA internal standard in both instances were then compared. The mean recovery of MP determined accordingly was 70%.

Finally, Fig. 6 displays the mean concentrations of MP in 200-mg cat spinal cord samples as a function of dose at one hour after injection. The variability among animals may reflect differences in spinal cord blood flow or differences in the amount of body fat (i.e., peripheral uptake of the MP). Inter-assay variability does not seem to be a factor, since repeated assays of the same sample over several days showed negligible variation.

The procedure described in this report is accurate and sensitive. The method is currently being used to measure the levels of MP in plasma and spinal cord after single large intravenous doses of MP for the purpose of providing a pharmacokinetic correlation with previously reported pharmacological data [1—5].

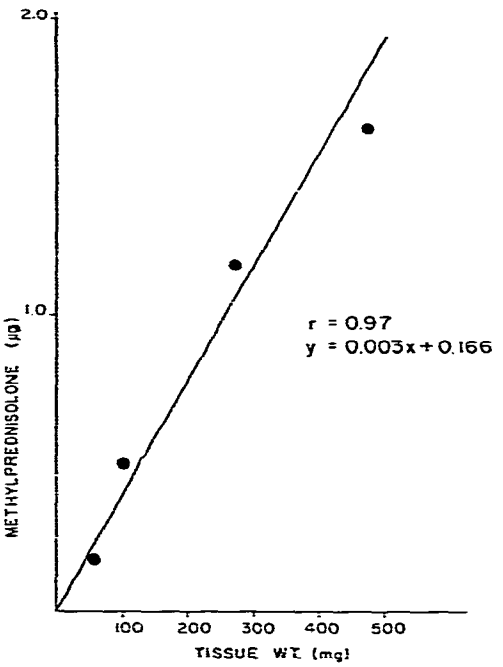

Fig. 5. Linearity of the MP content in spinal cord tissue following an intravenous injection of 90 mg/kg MP as a function of spinal cord sample size. Injection volume was 100 µl. All samples were run in duplicate.

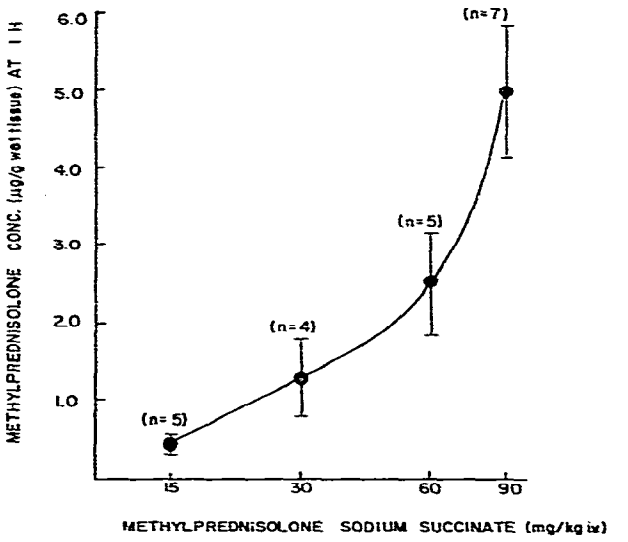

Fig. 6. Concentrations of MP in cat lumbar spinal cord samples at 1 h after intravenous administration of the dose of MP sodium succinate indicated. Values represent mean ± S.E. of duplicate determination from the number of animals indicated.

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REFERENCES

1 E.D. Hall and T. Baker, Exp. Neurol., 63 (1979) 476.
2 E.D. Hall, Pharmacologist, 22 (1980) 297.
3 P.A. McGinley, E.B. Truitt and E.D. Hall, Fed. Proc., Fed. Amer. Soc. Exp. Biol., 40 (1981) 299.
4 E.D. Hall and J.M. Braughler, Exp. Neurol., 73 (1981) 321.
5 J.M. Braughler and E.D. Hall, Brain Res., 219 (1981) 464.
6 M.D. Smith, J. Chromatogr., 164 (1979) 129.
7 F.J. Frey, B.M. Frey, A. Greither and L.Z. Benet, J. Pharmacol. Exp. Ther., 215 (1980) 287.
8 J.Q. Rose and W.J. Jusko, J. Chromatogr., 162 (1979) 273.
9 I.T. Agabeyoglu, J.G. Wagner and D.R. Kay, Res. Commun. Chem. Pathol. Pharmacol., 28 (1980) 163.
10 F.J. Frey, B.M. Frey and L.Z. Benet, Clin. Chem., 25 (1979) 1944.
11 R.I. Henkin, A.G.T. Casper, R. Brown, A.B. Harlan and F.C. Bartter, Endocrinology, 82 (1968) 1058.