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ISOLATION OF CORTISOL FROM THE BRAIN OF THE RHESUS MONKEY

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SUMMARY

Cortisol has been isolated from the brain of the rhesus monkey. The amounts per gram of wet tissue averaged 0·55 ± 0·06. This is in contrast to levels as high as 1·9 μg/g previously reported in human brain. Identification included infrared spectrometry.

INTRODUCTION

THE ISOLATION of cortisol from extracts of human brain was reported previously from this laboratory[5]. Tetrahydrocortisol and the 20β-reduced form of cortisol were also found. In this paper we describe the isolation of cortisol from extracts of rhesus monkey brain. The amounts found averaged 0·55 μg/g (wet weight) of tissue. Identification included infrared spectrometry.

MATERIALS AND METHODS

The monkey brain was removed within minutes after death of the animal by anesthetic overdose. The top of the skull was sawn off and the whole brain was removed, drained of excess fluid and blood and frozen until extracted. The tissue from 7 monkey brains was pooled and extracted for isolation of sufficient cortisol for identification.

The brains were weighed and 1/10 vol./wt. of distilled water was added. This was homogenized in a Waring blender followed by precipitation of protein through addition of 5 vol. of acetone. The precipitate was filtered and washed 3 times with resuspension with acetone and once with methanol. The combined solvents were evaporated *in vacuo* down to the aqueous residue.

The aqueous residue was extracted 3 times with equal volumes of 1 : 1 (v/v) chloroform–ethyl acetate mixture. The solvents were combined and evaporated *in vacuo*. No washings were performed. The residue was dissolved in hexane and the desired steroids extracted by washing 3 times with an equal volume of 70% methanol. The methanol was evaporated. The water was extracted with chloroform–ethyl acetate, 1 : 1 (v/v). The solvents were evaporated. The hexane fraction removed about 80% of the lipids.

The extract was subjected to paper chromatography on washed Whatman No. 1 filter paper (6 in. wide) in the system toluene–propylene glycol for one day for further lipid removal. The effluent was evaporated and the lipid residue was rechromatographed in the same system. This was repeated twice. After drying the strips the chromatograms were cut up and eluted with methanol and eluate from the three chromatograms combined. The methanol was evaporated.

The residue was then subjected to paper chromatography as before but continued for 5 days for separation of the polar corticoids by collecting 15–20 ml/½ in., as previously described[6]. The cortisol zone was located by its absorption of ultraviolet light and eluted. This was acetylated with pyridine and acetic anhydride, 2 : 1 (v/v), overnight at room temperature. After evaporation of the reagents the acetylated cortisol was purified by chromatography on washed Whatman No. 1 paper in the system toluene–propylene glycol with development for 24 hr. The acetate was further purified by chromatography on 1 cm × 10 cm silica gel columns. Sequential elution with benzene, benzene–ethyl acetate, 1 : 1 (v/v), and ethyl acetate was carried out. The acetate was found in the benzene–ethyl acetate eluate.

Spectrometry in sulfuric acid. The acetate was dissolved in concentrated sulfuric acid, kept at room temperature in the dark for 2 hr and then scanned in a Beckman DU spectrophotometer.

Infrared spectrometry. The acetate was mixed with potassium bromide and pressed into a macro-pellet and scanned in a Perkin–Elmer 421 infrared spectrometer.

RESULTS AND DISCUSSION

Figure 1 shows the pattern of the separation of the steroids on the paper chromatogram. Cortisol is readily seen by the absorption of ultraviolet light and positive reaction to blue tetrazolium, which are characteristic of cortisol. A summary of the qualitative tests performed to characterize the isolated cortisol is given in Table 1. The usual tests including spectrometry in sulfuric acid were

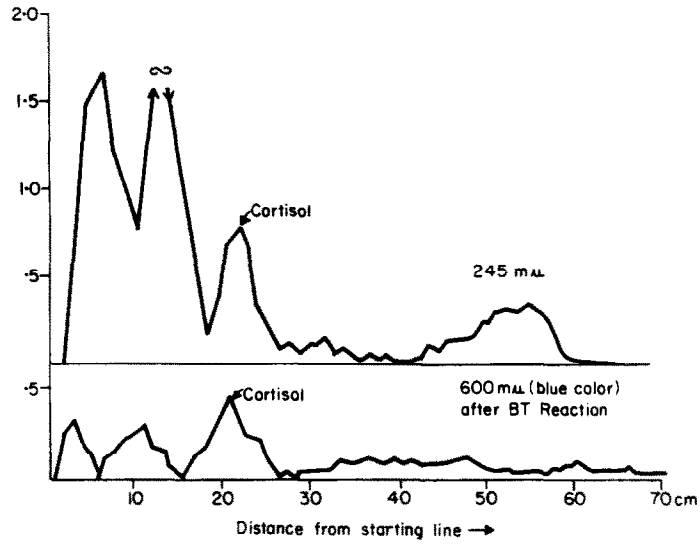

Fig. 1. Chromatographic absorption pattern of an extract of monkey brain. Half-inch wide paper strips were scanned directly in a Beckman DU spectrophotometer. The upper curve shows absorption pattern of the untreated paper chromatogram scanned at 245 mμ while the lower curve is the same strip after treatment with alkaline blue tetrazolium (BT) but scanned at 600 mμ.

Table 1. Identification of cortisol from monkey brain

Property	Isolated	Reference cortisol
Mobility with respect to DOC in toluene–propylene glycol paper chromatogram	0·017	0·017
Mobility of acetate in same system	0·14	0·14
Fluorescence in sulfuric acid	Green	Green
Reaction with blue tetrazolium	+	+
Absorption of ultraviolet light at 240 mμ	+	+
Maxima, spectrum in sulfuric acid of acetate	240 286 395 460	240 286 395 460
Infrared spectrum	Identical to reference cortisol (see Fig. 2)	

all confirmatory. The infrared spectrum of the acetate performed in KBr pellets was identical to that of the reference cortisol acetate (Fig. 2).

The most important feature of the isolation procedure described herein is the repeated rechromatography of the effluent of the initial paper chromatograms. This has been necessary because it is difficult to separate steroidal material from the large quantities of cholesterol present. By repeated chromatography it was possible to remove the cortisol in quantity suitable for identification. Solvent partition to separate the cholesterol was unsuccessful due to the large quantity of cholesterol present. The cholesterol appears to occlude the other steroids.

The amounts of cortisol found by this procedure in five monkey brains was $0{\cdot}55 \pm 0{\cdot}06$ μg/g of tissue. This is in contrast to the level of $1{\cdot}9$ μg/g found in this laboratory for the cortisol content of human brains. Whether this represents the true concentration of this corticoid can be answered when more efficient extraction procedures are developed. Cortisol metabolites (the 20-reduced form and the ring A-reduced form) were also indicated in the extracts. At present confirmation of identity awaits isolation of more material.

The cholesterol/cortisol ratio approximated 3×10^5. This, along with the large amounts of other lipids present, makes the use of thin layer chromatography unsatisfactory for preliminary separations. The lipids overloaded the thin layer chromatograms and separation was not possible. After the separation of the cortisol on paper further confirmation of the identity of cortisol was obtained on thin layer chromatograms. In the solvent system benzene–ethanol 8:2(v/v) cortisol

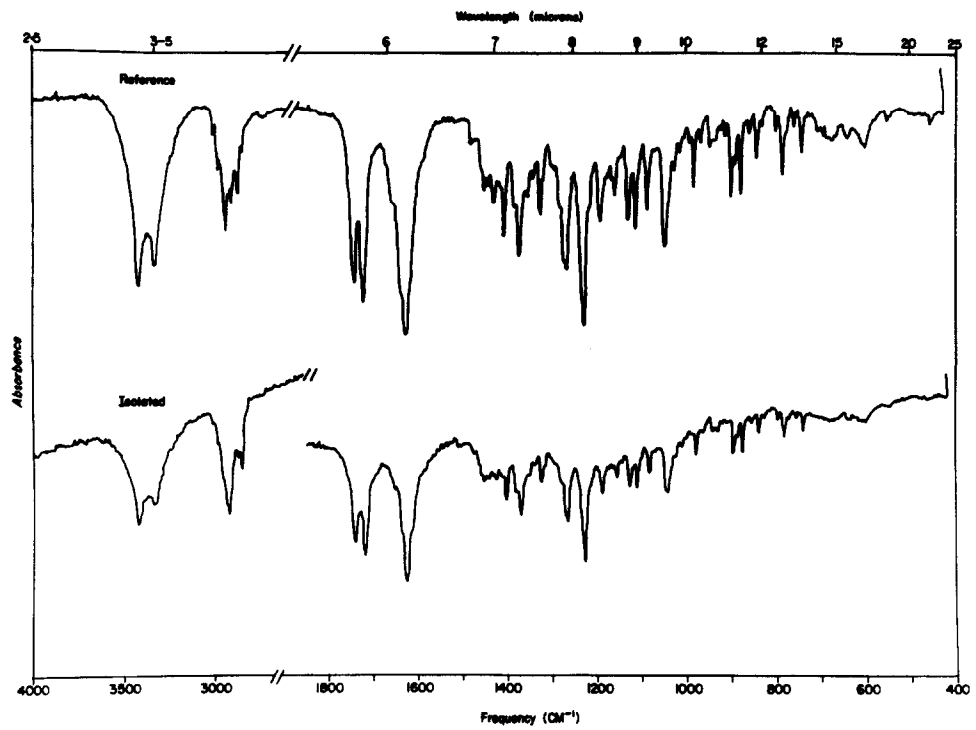

Fig. 2. Infrared spectra of the acetate of cortisol isolated from monkey brain. Also shown is the reference cortisol acetate. Spectrometry performed with Perkin–Elmer 421 using KBr pellets.

had an R_f of 0·54 which was the same as that of the reference cortisol on Silica gel G chromatograms 250 μ thick.

Nerve tissue, as well as brain tissue, from human subjects contains large quantities of cortisol. Concentrations appear to be higher than that found in the peripheral blood. At present the role of corticosteroids in the brain is not known.

Woodbury[6] and Dobbing[1] have reviewed some of the steroidal aspects of the brain. More recently Sholiton *et al.*[4] and Grosser[2,3] have shown that steroid metabolism is possible in the brain.

ACKNOWLEDGEMENTS

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REFERENCES

1. J. Dobbing: *Physiol. Rev.* **41** (1961) 130.
2. B. I. Grosser: *J. Neurochem.* **13** (1966) 475.
3. B. I. Grosser and E. L. Bliss: *Steroids* **8** (1966) 915.
4. L. J. Sholiton, R. T. Marnell and E. E. Weck: *Steroids* **8** (1966) 265.
5. J. C. Touchstone, M. Kasparow, R. Hughes and M. R. Horwitz: *Steroids* **7** (1966) 205.
6. D. M. Woodbury: *Pharmacol. Rev.* **10** (1958) 275.