ENDOCRINE RESEARCH, 26(4), 531-535 (2000)

CORTICOSTEROID PRODUCTION BY FETAL RAT HIPPOCAMPAL NEURONS

S.M. MacKenzie, C.J. Clark, M.C. Ingram,
*M. Lai, *J. Seckl, **C.E. Gomez-Sanchez,
R. Fraser, J.M.C. Connell, E. Davies.
MRC Blood Pressure Group, Western Infirmary, Glasgow, UK.
*Molecular Medicine Centre, Western General Hospital, Edinburgh, UK.
**Department of Internal Medicine, University of Missouri, Columbia, MO, USA.

ABSTRACT

11β-hydroxylase and aldosterone synthase catalyse the final stages of corticosterone and aldosterone synthesis respectively. Previously, we established that they are expressed in the rat brain, particularly the cerebellum and the hippocampus. Primary cultures of fetal rat neurons were studied. RT-PCR and immunohistochemistry established that neurons express 11β -hydroxylase and aldosterone synthase mRNAs and protein. After incubating the cells with 10μM DOC for 24 hours, medium was analysed for aldosterone and corticosterone. Median % conversion of DOC to corticosterone was 7.6% compared to 0.4% in controls. Median % conversion of DOC to aldosterone was 6.2% compared to 0.06% in controls. Corticosteroids mediate a number of functions of mammalian brain, including blood pressure homeostasis, salt appetite and neuronal excitability. Local production of these steroids could have significant effects on these processes.

INTRODUCTION

Recent evidence suggests that corticosteroid synthesis is not confined to the adrenal cortex but also occurs in other tissues, notably the vascular system and the brain (1-3). There is now firm evidence that the genes CYP11B1 and

Copyright © 2000 by Marcel Dekker, Inc. www.dekker.com

CYP11B2 encoding 11β-hydroxylase and aldosterone synthase respectively are transcribed in the rat brain to form the enzyme protein (4). Expression of the genes is localised to the Purkinje cells of the cerebellum and the dentate gyrus and pyramidal cells of the hippocampus.

The genes encoding the enzymes catalysing earlier reactions in the conversion of cholesterol to active corticosteroids are also expressed in these regions. Local de novo synthesis from cholesterol is therefore possible. Since low dose corticosteroid administration to the brain has marked effects on blood pressure and other aspects of metabolism (5), local synthesis may be physiologically important. The following study was designed to test whether aldosterone and corticosterone are synthesised in rat neural tissue.

METHODS

Preparation of primary rat fetal hippocampal neuronal cultures. Cultures were prepared from embryonic day 18 Wistar rats. Hippocampi were dissected out (6). Tissue was washed three times in Hanks balanced salt solution (Life Technologies) containing HEPES buffer, pH7.4, and then trypsinised (1mg/mL) for 10 minutes at 37° and dissociated by trituration through a 25-gauge sterile needle. Cells were seeded at a density of $2x10^6$ cells per 6 well poly-L-lysine coated tissue culture plate (Becton Dickinson) or $4x10^5$ cells per 8 well culture slide (Becton Dickinson) in Neurobasal medium containing B27 growth supplement and 0.5mM L-glutamine and grown in a humidified incubator at 37°C/5% CO_2.

The next day, cytosine arabinoside (5μM) was added to the medium to inhibit glial proliferation. Cells were left to grow for 7 days before experiments were carried out. Cultures typically contained less than 5% glia as determined by immunohistochemical staining with glial fibrillary associated protein (GFAP) antibody.

Cell incubations. 24 hours prior to incubation with DOC, cell medium was replaced with Neurobasal medium containing N2 supplement rather than B27, as

the latter contains low levels of corticosterone. Cells were incubated with 2mL of 10μM DOC in Neurobasal medium (GibcoBRL, Paisley, UK) with 1% N-2 supplement and 0.1% gentomycin. Control medium omittted DOC. Medium was removed after 24 hours and stored at -20°C. Cells were suspended in PBS and retained for protein measurement using the Bio-Rad protein assay kit (Bio-Rad, Hemel Hempstead, UK).

Steroid measurement. 25,000cpm of ^{3}H-corticosterone or ^{3}H-aldosterone was added to aliquots of incubation medium removed from replicate samples. Steroids were extracted with freshly-distilled dichloromethane and the extract washed with water and evaporated to dryness at 37° under nitrogen. Residues were chromatographed on paper using solvent system B5 for aldosterone and B1 for corticosterone (7) and appropriate steroid regions eluted in methanol after location by isotope scanning. Aldosterone samples were evaporated to dryness at 37° under nitrogen and the redissolved in radioimmunoassay diluent (Diagnostic Products Ltd, Gwynedd, UK) Recovery was assessed by measurement of ^{3}H content. Aldosterone was measured by direct radioimmunoassay (Diagnostic Products Ltd) and corticosterone by radioimmunoassay after partial chromatographic purification (8).

GCMS identification of aldosterone. 1mL of aldosterone was extracted with 6mL dichloromethane. This was washed with lmL water and evaporated to dryness and the residue chromatographed using the B5 solvent system. Aldosterone was located by UV of parallel standard and eluted in 5mL methanol, then evaporated under nitrogen. Samples were derivatised first by incubation with 0.5mL of 0.5M $H10_4$ in 1% pyridine at room temperature in the dark for 1½ hours. 1mL of water added, then the steroid was extracted with 6mL methylene choloride.

This was washed with 1mL water and then evaporated to dryness under nitrogen. 30μL of heptafluorobuutyric anhydride and 30μL of toluene were added to the residue and incubated at 60°C for 30 minutes. This was evaporated to dryness. 1mL hexane was added to the residue. Aldosterone standard was

derivatised in the same way. 1μL was injected on to GCMS (Varian Saturn GCMS System, Varian, Walnut Creek, California, USA).

RESULTS

The median conversion of 10μM DOC to corticosterone was 7.6% (range 0.1 - 20.4%, n = 10), compared to 0.4% in the control incubations. The median conversion of 10μM DOC to aldosterone was 6.2% (range 0.03 - 14.2%, n = 10), compared to 0.06% in the control incubation. The derivative of aldosterone extracted from the medium of the incubated fetal neuronal cells had a retention time of 37.9 minutes on capillary gas chromatography, identical to that of the derivative prepared from authentic standard aldosterone. The mass spectra of sample and standard were also identical.

DISCUSSION

These results confirm and extend the evidence of significant corticosteroid synthesis by neural tissue. The wide variation in the rate of conversion of DOC to aldosterone and corticosterone is likely to have been due to the extreme sensitivity of this cell type to handling. Previous studies using homogenates or minces of specific regions of the brain showed clear evidence of corticosteroidogenesis. These preparations, however, contain both neurons and associated glia (3). The current study shows unequivocally the steroidogenic potential of neurons, albeit fetal neurons. It is not possible from these experiments to estimate the potential contribution of this local synthesis to local tissue concentrations since systemic corticosteroids freely enter the brain.

From molecular bioloogy studies, the rates of CYP11B1 and CYP11B2 expression in the brain are much lowers than in the adrenal cortex (4). However, very small increments in the local concentration, for example, of aldosterone, have marked effects on systemic blood pressure (5). Similarly, it is not known whether the systems controlling neural and adrenal corticosteroidogenesis are the

same or different. Upon this in particular rests the physiological and clinical significance of local synthesis.

REFERENCES

1. Hatakeyaman H, Miyamori I, Takeda Y, Yamamoto H, Mabuchi H. Biochem Mol Biol Int. 40: 639 - 645.

2. Silvestre J S, Robert V, Heymes C, Aupetit-faisant B, Mouas C, Moalic J M, Swynghedauw B, Delcayre C. 1998. J. Biol Chem 237: 4883 - 4891.

3. Gomez-Sanchez CE, Zhou M, Cozza E N, Morita H, Foeking MF, Gomez-Sanchez EP. 1997. Endocrinology 138: 3369 - 3373.

4. MacKenzie S M, Clark C J, Fraser R, Gomez-Sanchez C E, Connell J M C, Davies E. 2000. J. Mol Endocrinol 24: 321 - 328.

5. Gomez-Sanchez EP. 1997. Frontiers Newoendocrinol 18: 440 - 462.

6. Banker G, Goslin K. 1991. Rat hippocampal neurons in low density culture. In: Banker G, Goslin K (eds) Culturing Nerve Cells. MIT Press: Cambridge Mass. Pp 251 - 282.

7. Bush I E. 1952. Biochem J. 50: 370 - 378.

8. Fraser R, Guest S, Young J. 1973. Clin Sci Mol Med 45: 411 - 415.