A COMPARATIVE STUDY OF HISTONE ACETYLATION IN NEURONAL AND GLIAL NUCLEI ENRICHED RAT BRAIN FRACTIONS

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1. Introduction

Neuronal chromatin templated transcription is much less restricted than that of oligodendro- and microglia [1,2]. These differences in chromatin template restriction are connected with differences in chromatin composition [3,4] and structure [5,10] and may be related to different enzymatic modifications of chromosomal proteins such as the previously described higher phosphorylation of neuronal nonhistone proteins [4].

Since the basic chromosomal proteins are certainly involved in determining chromatin structure [6] and in restricting the template capacity of DNA for in vitro synthesis [7,8] we were interested in the question whether and in what way nuclear histone transacetylases and deacetylases contribute to the structural and functional differences between neuronal and glial chromatin.

In the present paper we report on the higher acetylation of 'neuronal' chromatin-bound histones than 'glial' histones, on the very low deacetylation of histones by both the 'neuronal' and 'glial' nuclei and on the effect of in vitro histone acetylation on endogenous RNA polymerase activities in 'neuronal' and 'glial' nuclear fractions.

2. Methods

2.1. *Isolation of cell nuclei*

Rat brain nuclei were isolated from female Wistar rats (150-170 g body weight) as described elsewhere [9]. Neuronal (N) and glial (G) enriched nuclear fractions were separated from isolated rat brain nuclei by discontinuous sucrose density gradient centrifugation [4,9]. The nuclear fractions obtained corresponded morphologically to those described previously [4,9] and characterized by [5,10,11].

2.2. *Histone acetylation and deacetylation*

Histones were acetylated up to maximum values by incubating neuronal or glial nuclei enriched fractions (250 μg DNA) with 9.8 μCi [^{3}H] acetylcoenzyme A (spec. act. 187 mCi/mmol) in 0.32 M sucrose containing 3 mM $MgCl_2$ and 20 mM Tris–HCl pH 7.4 for 40 min at 37°C. Thereafter aliquots of the incubation assay were pipetted into ice-cold H_2SO_4 up to a final concentration of 0.4 N. Histones were extracted from the aliquots as described previously [12,19]. For determination of acetate retention in histones an excess of unlabeled acetylcoenzyme A was added at 40 min of incubation and the reaction was further incubated for 60 min and stopped by adding ice-cold H_2SO_4 up to 0.4 N.

2.3. *Gel electrophoresis and radioanalysis of histones*

Equal amounts of histones dissolved in 0.9 N acetic acid were separated by polyacrylamide gel electrophoresis in the presence of 3.125 M urea at 1 mA per 13.5 × 0.5 cm tube for 7 hr according to Panyim and Chalkley [13]. Gels were stained with 0.1% amido-black 10 B in 7% acetic acid containing 20% ethanol destained in 7% acetic acid with 35% ethanol and scanned at 600 nm using a Gilford spectralphotometer model 240 with scanning attachment. After cutting the gels into 1 mm slices using the apparatus described elsewhere [14] gel slices were depolymerized by 30% H_2O_2 and dissolved in Bray's scintillation solution [15] containing 1% NCS (Nuclear Chicago Solubilizer,

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Table 1
Effect of preincubation of neuronal and glial enriched nuclear fractions with acetylcoenzyme A on endogenous RNA polymerase activities.

Nuclear fraction	[^{3}H]UMP incorporated (dpm × μg DNA^{-1})		
	No preincubation (*) preincubation with (+) and without (–) acetylcoenzyme A	Low ionic strength conditions	High ionic strength conditions
'Neuronal'	(*)	63.7 ± 2.1	306.3 ± 15.8
	(–)	59.2 ± 1.6	281.8 ± 11.3
	(+)	81.5 ± 1.3	379.8 ± 13.9
'Glial'	(*)	26.5 ± 1.2	158.7 ± 4.9
	(–)	24.3 ± 1.9	147.1 ± 3.1
	(+)	30.3 ± 1.7	192.1 ± 6.3

After incubating the 'neuronal' and 'glial' nuclear suspension (about 500 μg DNA) with 500 pmol acetylcoenzyme A under conditions as described in Methods for 3 min 0.2 ml (about 50 μg DNA) of the suspensions were transferred to the RNA polymerase assays which contained 16.6 μCi [^{3}H]UTP (spec. act. 80 mCi/mmol) and UMP incorporation into an acid precipitable product was determined as described elsewhere [12,19]. Each value represents the arithmetic mean ± S.E.M. from 3 experiments.

Amersham/Searle, Illinois, USA). Radioactivity was determined in a Packard Liquid Scintillation Counter model 3380 using external standardisation. The efficiency for ^{3}H was 26.4%. Incorporation of radioactivity into total histones was determined as described by Sekeris [16]. DNA and protein were determined by the method of Burton [17] and Lowry [18] respectively.

3. Results and discussion

The endogeneous nuclear RNA polymerase activities at high and low ionic strength conditions are 93 and 140% higher in our 'neuronal' than in the 'glial' fraction (table 1) which is in agreement with reports in literature [1,2]. The higher in vitro RNA synthesis in the neuronal type nuclei corresponds with a higher nuclear acetylation rate of 'neuronal' histones (fig.1) if compared to 'glial' histones. In the presence of an excess of acetylcoenzyme A the uptake capacity for [^{3}H]acetate in the 'neuronal' chromatin-bound histones is nearly twice as much as in the 'glial' histones. This is connected with a more rapid uptake of [^{3}H]acetate by 'neuronal' than by 'glial' histones during the first 20 min of incubation (fig.1 B) when acetylation

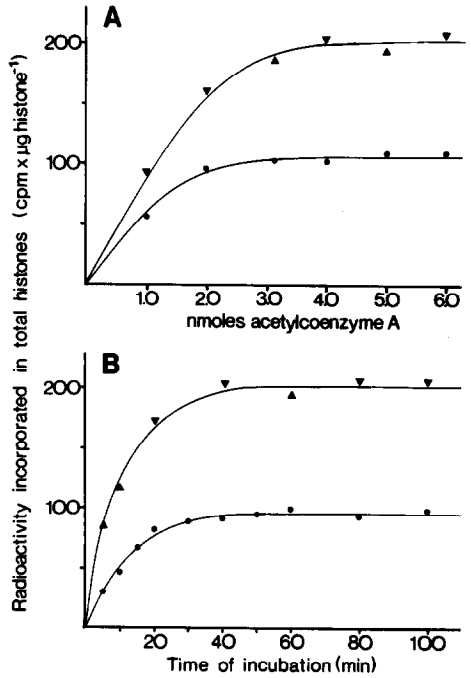

Fig.1. [^{3}H]acetyl incorporation into total histones by neuronal (▼——▼) and glial (●——●) nuclei enriched rat brain fractions. (A) Dependence on increasing amounts of [^{3}H]acetylcoenzyme A (spec. act. 187 mCi/mmol). (B) Dependence on incubation time.

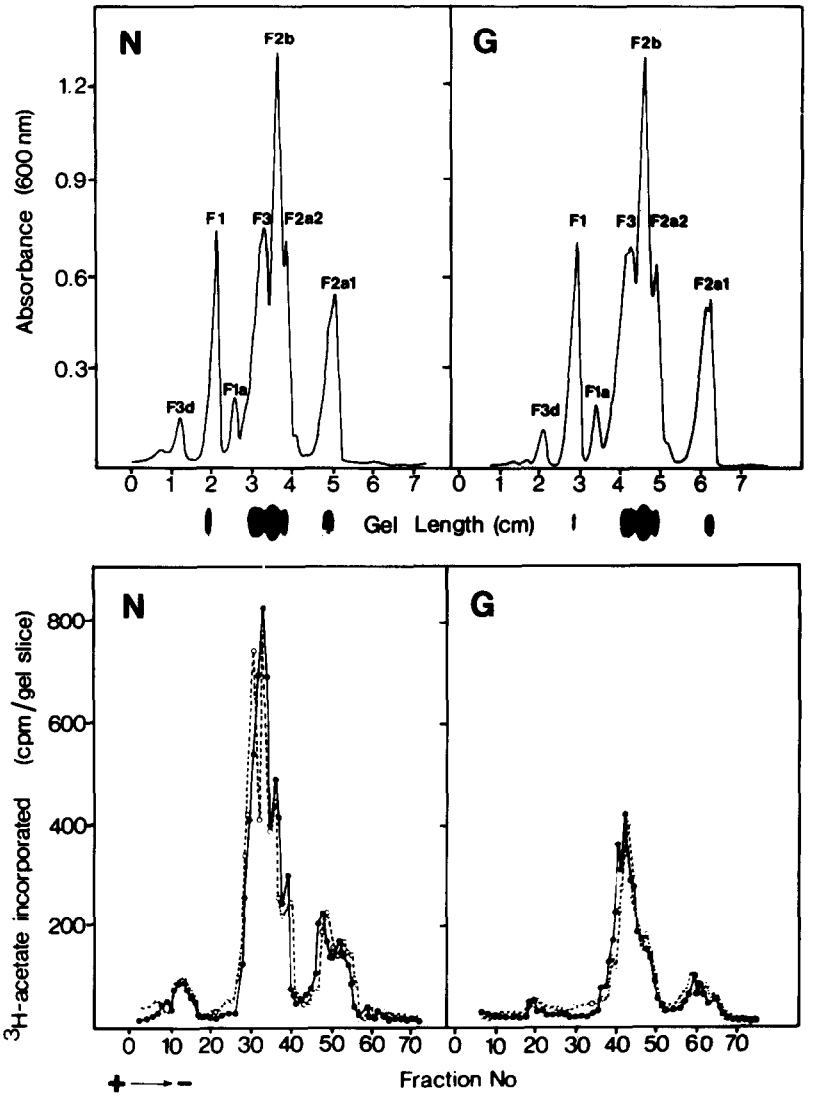

Fig.2. Acetylation and deacetylation of histone fractions from neuronal (N) and glial (G) enriched nuclei of rat brain. *Upper part*: Densitometric tracings and photographs of the banding profiles of 'neuronal' and 'glial' acetylated histones. *Lower part*: 50 μg histones acetylated up to maximal values by incubating the nuclear fractions with [^{3}H] acetylcoenzyme A for 40 min (•——•) and after further incubation for 60 min in the presence of an excess of unlabeled acetylcoenzyme A (o——o) were gelelectrophoretically separated, scanned, sliced and radioanalyzed as described in Methods.

occurs linearily with time. The [^{3}H] acetate taken up after 40 min of incubation is associated mainly with F3, F2b, F2a fractions of 'neuronal' as well as of 'glial' histones (fig.2).

The plateau values of acetate incorporation into 'neuronal' and 'glial' histones after 40 min of incubation could result either from a relatively fast acetylation including a reiterated use of [^{3}H] acetate or from a relatively slow deacetylation or from both these processes. In order to differentiate between the three possibilities the histone-bound [^{3}H] acetate was chased in the presence of an excess of unlabeled acetylcoenzyme A for 60 min after histones had been saturated with [^{3}H] acetate (fig.2).

Interestingly none of the histone fractions did release its incorporated acetate during the 60 min chase period. This is different to rat liver where histone deacetylation occurred under the same experimental conditions [19]. At present it is difficult to give a sufficient explanation for the low deacetylation of chromatin-bound histones in the rat brain nuclear fractions. However, an artificial reduction of deacetylase activity due to the isolation procedure for 'neuronal' and 'glial' nuclei is not very likely since transacetylases, RNA polymerases [20], protein kinases [4] and enzymes responsible for nuclear amino acid incorporation [9] are highly active in these nuclear populations. Since no measurable deacetylase activity was found neither in 'neuronal' nor in 'glial' nuclei as early as after 3 min of incubation a loss of deacetylase activity due to the length of the chasing period can be excluded.

The biological meaning of 'neuronal' and 'glial' histone acetylation for in vitro RNA synthesis is reflected in table 1. Preincubation of neuronal enriched nuclei with acetylcoenzyme A for 3 min is connected with an acetate uptake into chromatin-bound histones of 0.275 pmol acetate/μg DNA X min and leads to an increase of UMP incorporation of 0.185 pmol UMP/μg DNA X min under high ionic strength conditions and in the presence of Mn^{2+} ions and to an increased incorporation of 0.041 pmol UMP/μg DNA X min under low ionic strength conditions and in the presence of Mg^{2+} ions. Under identical conditions uptake of 0.099 pmol acetate/μg DNA X min into 'glial' histones corresponds to an increase of incorporation of 0.085 pmol UMP/μg DNA X min by high ionic strength and Mn^{2+}-dependent nuclear RNA polymerase and to an increase of UMP incorporation of 0.011 pmol/μg DNA X min catalyzed by low ionic strength and Mg^{2+}-dependent RNA polymerase. (UMP values calculated from table 1). So a positive correlation seems to exist between the extent of acetylation of chromatin-bound histones and the extent of chromatin templated UMP incorporation by endogenous RNA polymerases in 'neuronal' nuclei compared to that in 'glial' nuclei. The data of table 1 and fig.1 further suggest that acetylation of a few acetate binding sites in chromatin-bound 'neuronal' and 'glial' histones leads to an increased transcription of chromatin-DNA whereas the higher saturation values of acetate binding to chromatin-bound 'neuronal' histones as compared to 'glial' ones (fig.1) may be connected with more acetyl binding sites and to the looser structures of the 'neuronal' chromatin.

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References

[1] Kato, T. and Kurokawa, M. (1970) Biochem. J. 116, 599–609.
[2] Austoker, J., Cox, D. and Mathias, A. P. (1972) Biochem. J. 129, 1139–1155.
[3] Tashiro, T., Mizobe, F. and Kurokawa, M. (1974) FEBS Lett. 38, 121–124.
[4] Fleischer-Lambropoulos, H., Sarkander, H.-I. and Brade, W. P. (1974) FEBS Lett. 45, 329–332.
[5] Nurnberger, J. I. and Gordon, M. W. (1957) in: Progress in Neurobiology (Waelsch, M., ed.). Vol. 2, pp.100–138, Harper and Row, New York.
[6] Bonner, J. and Garrard, W. T. (1974) Life Sciences 14, 209–221.
[7] Allfrey, V. G. (1971) in: Histones and Nucleohistones (Phillips, D. M. P., ed.) pp.241–294, Plenum Press, New York.
[8] Huang, R. C. and Bonner, J. (1962) Proc. Natl. Acad. Sci. USA, 48, 1216–1222.
[9] Fleischer-Lambropoulos, H. and Reinsch, I. (1971) Hoppe-Seyler's Z. physiol. Chem. 352, 593–602.
[10] Glees, P. (1955) Neuroglia, Morphology and Funktion, Blackwell, Oxford.
[11] Burdman, J. A. and Journey, L. J. (1969) J. Neurochem. 16, 493–508.
[12] Sarkander, H.-I., Kemmerle, M. and Brade, W. (1974) Naunyn-Schmiedeberg's Arch. Pharmacol. 284, 39–53.
[13] Panyim, S. and Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337–346.
[14] Brade, W. and Dietz, H. (1973) Analyt. Biochem. 51, 641–645.
[15] Bray, G. A. (1960) Analyt. Biochem. 1, 279–285.
[16] Sekeris, C. E., Sekeri, K. E. and Gallwitz, D. (1967) Hoppe-Seyler's Z. physiol. Chem. 348, 1660–1666.
[17] Burton, K. (1956) Biochem. J. 62, 315–323.
[18] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[19] Sarkander, H.-I., Fleischer-Lambropoulos, H. and Brade, W. P. (1975) Biochem. Pharmacol., submitted for publication.
[20] Sarkander, H.-I., Fleischer-Lambropoulos, H. and Brade, W. P. (1975) Suppl. to Naunyn-Schmiedeberg's Arch. Pharmacol., in press.