EFFECTS OF POLYAMINES ON AMINO ACID INCORPORATION INTO PROTEIN BY CEREBRAL AND CEREBELLAR AS WELL AS "NEURONAL" AND "GLIAL" NUCLEI OF RAT BRAIN

H. Fleischer-Lambropoulos, H.-I. Sarkander and W.P. Brade

Institut für Pharmakologie der Freien Universität
D-1000 Berlin 33, Thielallee 69-73, Germany

Received February 2,1975

SUMMARY: The effects of polyamines on in vitro amino acid incorporation into rat cerebral, cerebellar, "neuronal" and "glial" nuclei have been found to be dose dependent. Maximal increase occurred at 0.1 mM spermine and 1 mM spermidine respectively, at higher concentrations of spermine and spermidine nuclear amino acid uptake by all systems examined was inhibited. These results suggest that polyamines may have a regulating function for nuclear amino acid incorporation in rat brain.

INTRODUCTION

Aliphatic polyamines such as putrescine, spermine and spermidine are assumed to be involved in vital cell functions such as synthesis of RNA (1,2) and protein (3-7). Although polyamines occur in relatively large amounts in the brain (8) and polyamine concentrations have been reported to vary in different brain regions (9), no clear information is available on their biological role in nervous tissue.

On the other hand functional and metabolic differences between the various cell types of the brain seem to be connected with differences in nuclear processes such as RNA polymerase activities (10,11), nonhistone phosphorylation (12) and amino acid incorporation (13).

As a first step towards elucidating the functional significance of polyamines in the nervous system, we have studied the effects of these substances on nuclear protein synthesis of neuronal and glial tissues.

MATERIALS and METHODS

Female Wistar rats (140-150 g), previously starved for 16 hrs, were killed by decapitation. The brains were rapidly removed and separated into cerebellum and cerebrum. All operations were carried out at 4°C.

Cerebral and cerebellar nuclei were isolated by means of hypertonic sucrose and Triton X-100 (13). Brain nuclei were fractionated into two different populations - "neuronal" and "glial" - by discontinuous sucrose density gradient centrifugation, as described elsewhere (12,13).

Polyribosomes from total brain were isolated by use of the non-ionic detergent Triton X-100 and high KCl concentration (0.24 M) according to Gielkens et al. (14).

Spermidine (Fluka, Switzerland) and spermine (Merck-Schuchardt, Germany) were dissolved in bi-distilled water, adjusted to pH 6.8 with HCl.

The assay for nuclear amino acid incorporation contained in a final volume of 0.5 ml: 0.1 ml nuclear suspension (300 µg protein); 0.07 ml (450 µg protein) of the 105 000 x g supernatant from rat brain; 0.002 ml pyruvate kinase (20 µg); 25 mM sodium phosphate buffer, pH 6.75; 5 mM sucrose; 20 mM glucose; 60 mM NaCl; 7.5 mM $MgCl_2$; 0.05 mM ATP; 1 mM GTP; 1.5 mM GSH; 3 mM phosphoenolpyruvate; 0.05 mM each of a mixture of 20 inactive amino acids with the exception of leucine; 5 µCi [^{3}H] leucine (specific activity: 36 Ci/mmole respectively 52 Ci/mmole; Amersham, England).

The assay for ribosomal amino acid incorporation contained in a final volume of 0.5 ml: 0.2 ml ribosomal suspension (770 µg RNA); 0.08 ml (550 µg protein) of the 105 000 x g supernatant of rat brain; 0.015 ml pyruvate kinase (150 µg); 40 mM Tris, pH 7.8; 7.5 mM $MgCl_2$; 1 mM ATP; 0.25 mM GTP; 1 mM GSH; 10 mM phosphoenolpyruvate; 60 mM KCl; 0.05 mM each of a mixture of 20 inactive amino acids, with the exception of phenylalanine; 0.01 mM polyuridylic acid; 1.5 µCi [^{14}C]phenylalanine (specific

activity: 415 Ci/mmole; Amersham, England).

For preparation of the 105 000 x g supernatant and for further details of incubation see elsewhere (13).

After 15 min of incubation at 37°C, trichloroacetic acid-insoluble radioactivity for both systems was determined in a 0.1 ml aliquot using the paper-disk method of Mans and Novelli (15). Radioactive samples were counted in Bray's scintillation fluid (16) using a Packard TriCarb liquid scintillation Counter (model 3380) with automatic standardization. DNA was determined by the diphenylamine reaction (17), RNA by the orcinol method (18) and protein according to Lowry et al. (19).

RESULTS and DISCUSSION

We have reported in previous work on the higher amino acid uptake by "neuronal" than by "glial" rat brain nuclei (13). With regard to the question of whether and in what way polyamines effect nuclear peptide synthesis in different brain cells, amino acid incorporation into rat cerebral and cerebellar nuclei, respectively into "neuronal" and "glial" nuclear populations, was measured in the presence of increasing polyamine concentrations.

Spermine and spermidine exhibit dose dependent effects on the in vitro amino acid uptake by rat brain nuclei (Fig. 1) : Maximal stimulation of [^{3}H]leucine incorporation by cerebral as well as by cerebellar nuclei occurred at 0.1 mM spermine and 1 mM spermidine respectively. At higher concentrations the polyamines inhibit the nuclear amino acid uptake in cerebrum and cerebellum.

The physiological meaning of the stimulatory effect of polyamines on nuclear amino acid incorporation is suggested by the following findings: Those spermine and spermidine concentrations found to be stimulatory on nuclear amino acid uptake

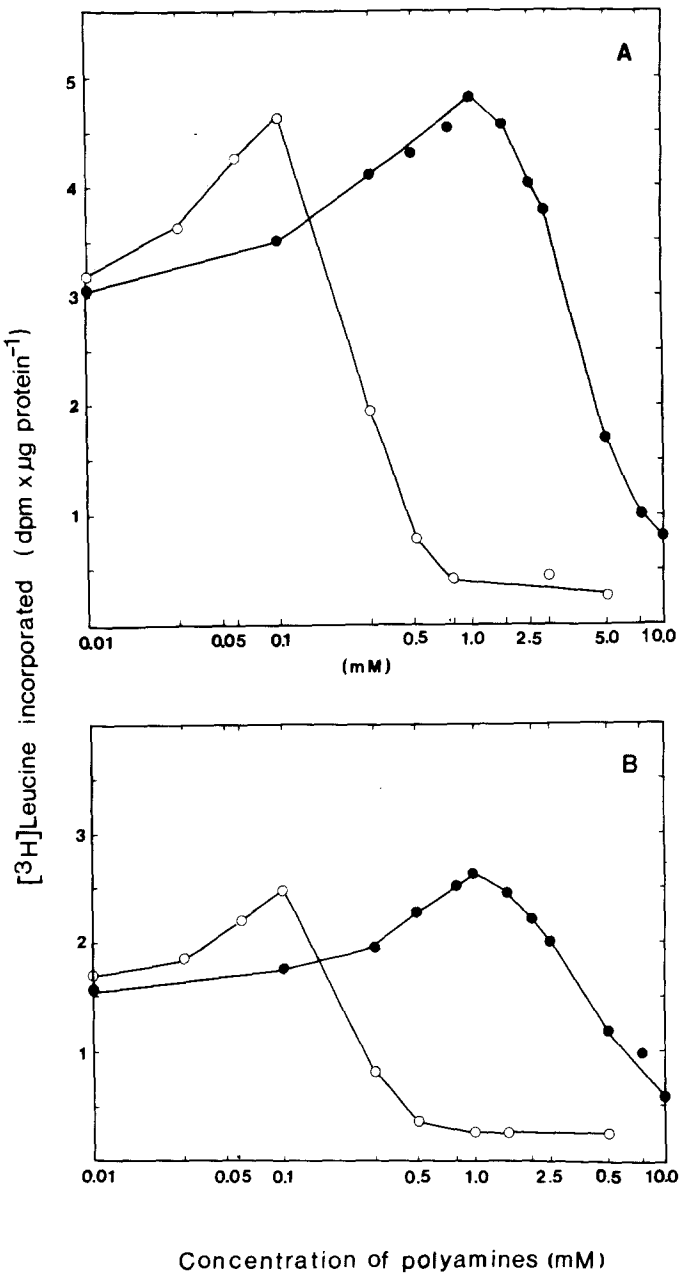

Fig. 1 Effect of spermidine (●-●) and spermine (○-○) on incorporation of radioactivity by incubating rat cerebral (A) and cerebellar (B) nuclei with 5 µCi [^{3}H] leucine (spec. act.: 36 Ci/mmole) as described under Materials and Methods. Values are means of quadruplicate determinations.

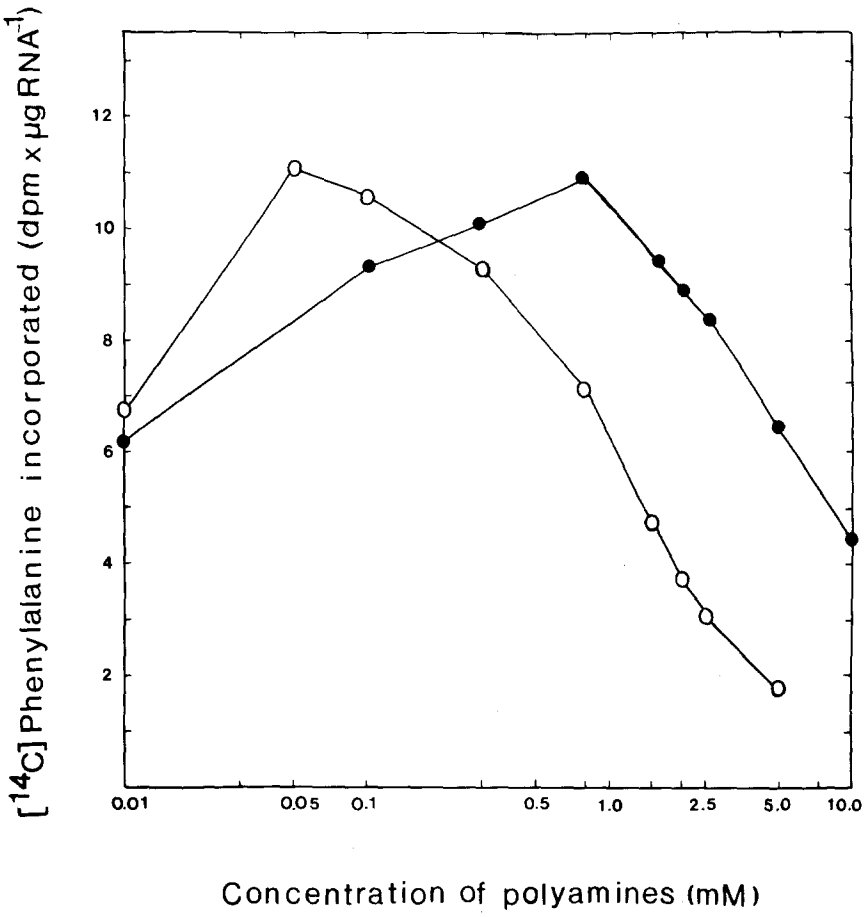

Fig. 2 Effect of spermidine (● -●) and spermine (o -o) on incorporation of radioactivity by incubating polyribosomes of rat brain with 1.5 µCi [^{14}C]phenylalanine (spec. activity 415 Ci/mmole) as described under Materials and Methods. Values are means of quadruplicate determinations.

(Fig. 1) are in the range of the polyamine concentrations occurring in rat cerebral cortex and cerebellum (9). Similar concentrations, i.e. 0.25 mM spermine and 2.5 mM spermidine have been shown to enhance in vitro RNA synthesis (20). Furthermore, in rat brain both nuclear and ribosomal [^{3}H]leucine uptake are increased maximally by nearly the same spermine and spermidine concentrations (Fig. 1,2). This might suggest that in rat brain

TABLE I Effect of spermidine on incorporation of radioactivity by incubating "neuronal" and "glial" nuclei of rat brain with 5 uCi ^{3}H leucine (spec. activity: 52 Ci/mmole).

Spermidine	dpm / mg protein	
	"neuronal" nuclei	"glial"
None	5729	1724
1.0 mM	7664	2635
1.5 mM	6510	2241
7.5 mM	276	659

Isolation of "neuronal" and "glial" nuclei as described under Materials and Methods. Nuclear material was derived from 60 rat brains.
Values are arithmetic means of 4 incubation assays.

the mechanism of protein synthesis in the nucleus and on cytoplasmic ribosomes may have some common features.

There are significant differences in the basal level of amino acid uptake between the cerebral and cerebellar, respectively between "neuronal" and "glial" nuclear populations (Fig. 1, Tab. I). Independent of such differences the stimulatory effect of spermine and spermidine ranges between 40 % and 60 % in all amino acid incorporating systems examined, i.e. in brain ribosomes and in cerebral, cerebellar,"neuronal" and "glial" nuclei (Fig. 1,2, Tab. I).

Since multiple effects of polyamines - e.g. on RNA synthesis (1,2) and on ribosomal protein synthesis (1,3,5) have been attributed to their cationic nature (1), we tested the combined effects of various Mg^{2+} concentrations and of 1 mM spermidine, a concentration which stimulates maximally rat cerebral leucine

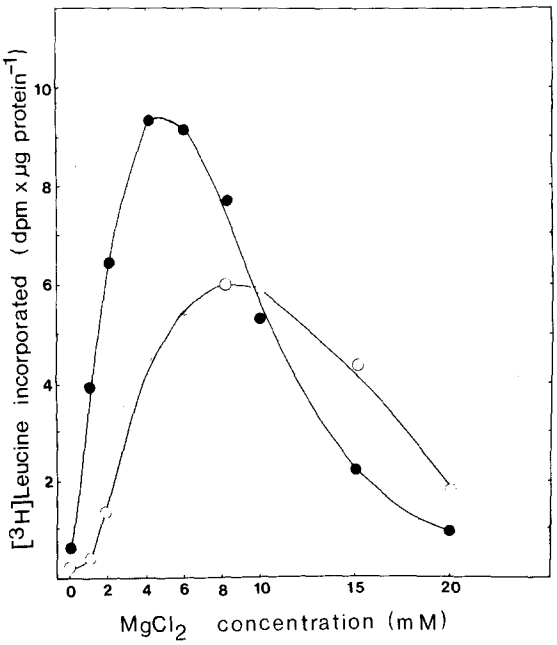

Fig. 3 Effect of 1 mM spermidine (● - ●) on incorporation of radioactivity by incubating cerebral nuclei with 5 µCi [^{3}H] leucine (spec. act. 52 Ci/mmole) at various Mg^{2+} concentrations; (○ - ○) no polyamines. Incubation conditions and determination of amino acid incorporation as described under Materials and Methods.
Values are means of quadruplicate determinations.

uptake at 7.5 mM Mg^{2+}. As can be seen from Fig. 3, omission of Mg^{2+} in the incubation assay leads almost to zero values of cerebral nuclear amino acid incorporation; this is in agreement with other reports on nuclear protein synthesis (13,21).
1 mM spermidine, a concentration which was found to stimulate maximally rat cerebral amino acid incorporation in the presence of 7.5 mM Mg^{2+}, cannot completely substitute for Mg^{2+}, but causes a shift of the Mg^{2+} concentration required for maximal amino acid uptake by rat cerebral nuclei from 7.5 mM to 4 mM Mg^{2+}. This is in accordance with results obtained from studies with bacterial and mammalian ribosomes (3,5,22) and is

supported by our findings that complete replacement of Mg^{2+} by polyamines in the sucrose solutions used for isolation of rat cerebral nuclei abolishes nuclear amino acid incorporation. Interestingly, the degree of leucine uptake observed with 1 mM spermidine, in the presence of Mg^{2+} concentrations suboptimal for amino acid incorporation without polyamines, is significantly higher than that seen in the absence of spermidine under optimal Mg^{2+} concentrations. This indicates that parallel to the sparing effect on Mg^{2+}, which may be connected to the cationic nature of polyamines, additional sites in nuclear protein synthesis are affected by spermidine.

Most recently Igarashi et al. (7) reported that polyamines play an important role in protein synthesis possibly by regulating formation and binding of aminoacyl-t-RNA to ribosomes. At present we cannot differentiate whether similar mechanisms are involved in the polyamine induced increase of rat brain nuclear amino acid incorporation. However, our findings suggest that rat brain nuclei do respond to physiological polyamine concentrations, which have been shown to vary in different brain regions.

Acknowledgements: The authors are indebted to Professor C.E. Sekeris for critical reading; thanks are also due to Miss I. Reinsch, Mrs.M. Kemmerle and B. Schröder for excellent technical assistance.

References

1. Tabor, H. and Tabor, C.W. (1972) Advances in Enzymology 36, 203-253.
2. Caldarera, C.M., Moruzzi, M.S., Rossoni, C. and Barbiroli, B. (1969) J. Neurochem. 16, 309-316.
3. Takeda, Y. (1969) Biochem. Biophys. Acta 182, 258-261.
4. Giorgi, P.P. (1970) Biochem. J. 120, 643-651.
5. Igarashi, K., Hikami, K., Sugawara, K. and Hirose, S. (1973) Biochem. Biophys. Acta 299, 325-330.

6. Igarashi, K., Takahashi, K. and Hirose, S. (1974) Biochem. Biophys. Res. Commun. 60, 234-240.
7. Igarashi, K., Sugawara, K., Izumi, I., Nagayama, C. and Hirose, S. (1974) Eur. J. Biochem. 48, 495-502.
8. Kremzner, L.T. (1970) Feder. Proc. 29, 1583-1588.
9. Snyder, S.H., Shaskan, E.G. and Harik, S.I. (1973) Polyamines in Normal and Neoplastic Growth, pp. 199-219, Raven press, New York.
10. Austoker, J., Cox, D. and Mathias, A.P. (1972) Biochem. J. 129, 1139-1155.
11. Thompson, R.J. (1973) J. Neurochem. 21, 19-40.
12. Fleischer-Lambropoulos, H., Sarkander, H.-I. and Brade, W.P. (1974) FEBS Lett. 45, 329-332.
13. Fleischer-Lambropoulos, H. and Reinsch, I. (1971) Hoppe Seyler's Z. Physiol. Chem. 352, 593-602.
14. Gielkens, A.L.J., Berns, T.J.M. and Bloemendal, H. (1971) Eur. J. Biochem. 22, 478-484.
15. Mans, R.J. and Novelli, G.D. (1961) Arch. Biochem. Biophys. 94, 48-53.
16. Bray, G.A. (1960) Analyt. Biochem. 1, 279-285.
17. Burton, K. (1956) Biochem. J. 62, 315-323.
18. Ogur, M. and Rosen, G. (1950) Arch. Biochem. 25, 262-276.
19. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) 193, 265-275.
20. Herbst, E.J., Byus, C.V. and Nuss, D.L. (1973) Polyamines in Normal and Neoplastic Growth, pp.71-90, Raven press, N.Y.
21. Dravid, A.R. and Wong, E. (1972) J. Neurochem.19, 2709-2725.
22. Takeda, Y. (1969) Biochem. Biophys. Acta 179, 232-234.