SYNAPTOSOMAL PROTEINS AND AXOPLASMIC FLOW: FRACTIONATION BY SDS POLYACRYLAMIDE GEL ELECTROPHORESIS

Peter MARKO*, Jean-Philippe SUSZ and Michel CUÉNOD
Brain Research Institute, University of Zurich, Zurich, Switzerland

and

**Friedrich Miescher Institut, Basel, Switzerland*

Received 28 July 1971

1. Introduction

The neuronal cell body is known to supply the axon and the nerve terminal with various materials which migrate through the axon at several different velocities. A perikaryal origin of an important part of the synaptic proteins has been suggested and substantiated [1].

Using the visual system of the pigeon as a model, it was shown that proteins labelled in one retina reach the optic nerve terminals in the contralateral optic lobe in at least two waves. The first, corresponding to the fast axoplasmic flow, has a peak at 24 hr while the second, having the characteristics of slow axoplasmic flow, has its maximum at 14 days [2–4]. Previous attempts to fractionate the synaptosomal proteins [5–8] have not dealt with the source of origin of these proteins, i.e. the problem was not investigated whether the proteins arrive with the fast or slow axonal flows or whether they are synthesized in the nerve endings. This report is concerned with the characterization, by sodium dodecyl sulfate (SDS) acrylamide gel electrophoresis, of the synaptic proteins migrating from the perikaryon.

2. Materials and methods

Each pigeon was injected intraocularly [2] with 50 μCi of ^{3}H-leucine (1-4,5-^{3}H-leucine, specific activity 22 Ci/mM) or 50 μCi ^{14}C-leucine (1-1-^{14}C-leucine, specific activity 62 mCi/mM, both Radiochemical Centre, Amersham, England) in 50 μl H_2O. Subcellular fractionation of the optic lobe's homogenate was performed by the slightly modified [2] method of Gray and Whittaker [9]. The fraction enriched in synaptosomes, containing about 1.5 mg protein, was solubilized in 200 μl of SUCK solution (1% SDS, 8 M urea, 0.01 M dithiotreitol and 0.05 M K_2CO_3) [10, 11] by gentle agitation at room temperature for 30 min and used immediately for the electrophoresis.

An ORTEC SLAB electrophoresis unit was used with a pulsed constant-power source. 0.1% SDS acrylamide discontinuous gradient gel was prepared according to Grossfeld and Shooter [12]. In each cell, 7.5 ml of 12% acrylamide, 7.5 ml of 9.6%, 6 ml of 7.5% and 3 ml of 4.2% were layered on top of each other and polymerized simultaneously. The stacking gel, 8 ml of 2.5% acrylamide, was then polymerized with light. 50 μl of synaptosomes solubilized in SUCK were applied on the stacking gel. Electrophoresis was performed in the discontinuous Tris glycine buffer system [12] using 300 pulses/sec at 120 V, 10 mA and 0.1 μF discharge capacitance. The proteins moved towards the anode at room temperature for 16 hr. The gels were fixed in concentrated acetic acid for 12 hr and stained with 1% amido black B in 7% (v/v) acetic acid for 3 hr. They were destained in 7% acetic acid and sectioned between successive bands, where mechanical separation was possible. The pieces of gel were solubilized [13] and counted in a Mark I Nuclear Chicago Scintillation Counter. The dpm were

calculated [14] with toluene standards. The amount of protein was determined according to Bramhall et al. [15].

3. Results and discussion

The synaptosomal proteins were completely extracted by the procedure used, the yield being 98%. All the material applied on the top of the gel migrated into it giving reproducible bands (fig. 1A).

In alternate sequence ^{3}H- and ^{14}C-leucine were injected into the same eye 14 and 1 days before sacrifice. In six experiments an average of 170 dpm was recovered on the control side, and on the experimental side the recovery was 990 dpm. The following pattern was observed: Although the radioactivity was distributed throughout the bands, the isotope injected one day before sacrifice preferentially labelled the slow moving bands, while the isotope injected 14 days before sacrifice labelled mainly the fast moving bands of the gel. This can be seen in fig. 1B which gives the distribution of radioactivity for each isotope and in fig. 1C showing their ratios. In both curves, the radioactivity of the control side was subtracted from that of the experimental side (fig. 1A) for each band individually, in order to exclude the backround activity [2, 16].

By analogy with continuous SDS polyacrylamide gel [11] one can infer that the molecular weights of the proteins in the bands decrease from the beginning to the end of the gel. Thus, the results suggest that the proteins migrating with fast axoplasmic flow (1 day) have higher molecular weights or are built from higher molecular weight subunits when compared with the proteins or their subunits labelled after a delay of 14 days. The latter ones represent the material migrating with the slow axoplasmic flow, although they may contain proteins from fast axoplasmic flow having a relatively slow turnover in the nerve endings or some material labelled by reutilization of isotope after catabolism. The results are in agreement with those of Karlsson and Sjöstrand [17], although these were not concerned with synaptosomes.

The splitting of the proteins into subunits during solubilization could randomize their distribution in the gel and thus be the reason for the overlap between ^{3}H- and ^{14}C-activity. However, after treatment with

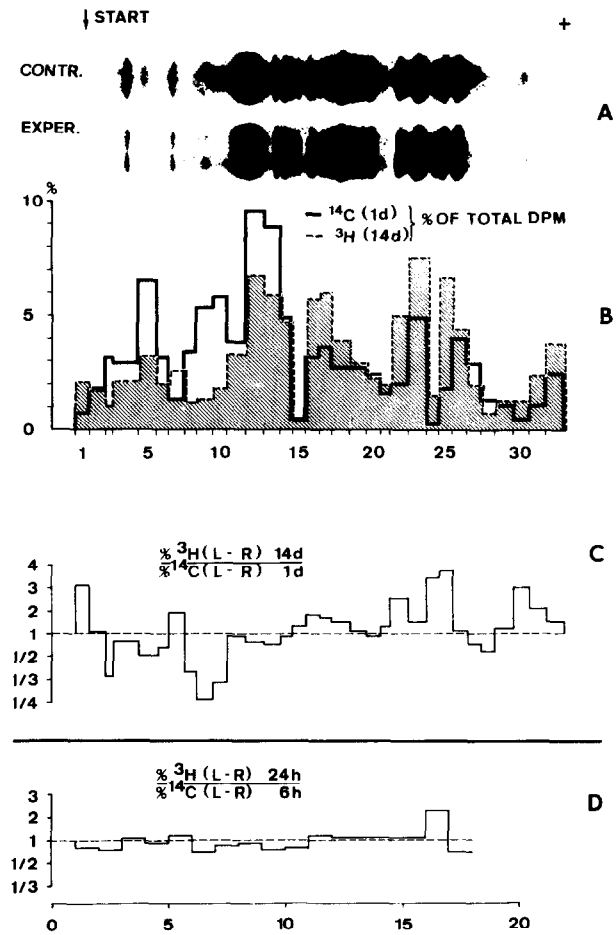

Fig. 1. SDS polyacrylamide gel electrophoresis of tectal synaptosomal proteins. (A)–(C) Injection of 50 μCi ^{14}C-leucine and 50 μCi ^{3}H-leucine in the right eye, 14 and 1 days, respectively, before sacrifice. (A) Gels of synaptosomal proteins from optic lobe corresponding to the injected eye (exper.) and to the control eye (contr.). (B) Distribution of activity difference (exper.–contr.) for each band expressed as % of total difference (640 dpm for ^{14}C-activity and 630 dpm for ^{3}H-activity). (C) Ratio ^{3}H-activity divided by ^{14}C-activity for each band from B. (D) The same as (C) from an experiment in which 50 μCi ^{14}C-leucine and 50 μCi ^{3}H-leucine were injected 6 and 24 hr, respectively, before sacrifice. Abscissa: band number.

colchicine, which is known to inhibit the axonal flows, the activity was not evenly reduced [18]. Consequently, it is unlikely that randomization during SDS extraction is the only cause for the broad distribution of activity.

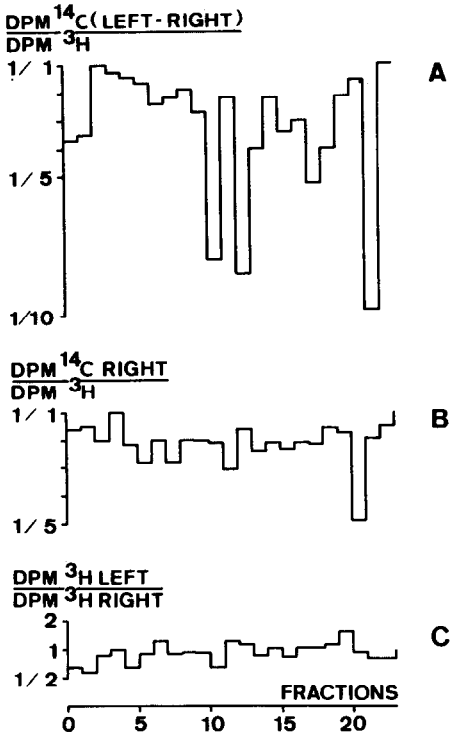

Fig. 2. SDS polyacrylamide gel electrophoresis of tectal synaptosomal proteins. Simultaneous injection, 24 hr before sacrifice, of 50 μCi ^{14}C-leucine in the right eye and 200 μCi ^{3}H-leucine intravenously. The total radioactivity in each gel was: ^{14}C-left 418 dpm, ^{14}C-right 235 dpm, ^{3}H-left 483 dpm and ^{3}H-right 504 dpm (A) Ratio of difference in ^{14}C-activity (exper.–contr.) divided by ^{3}H-activity for each band. (B) Ratio ^{14}C-activity divided by ^{3}H-activity on the control side. (C) Ratio ^{3}H-activity on the experimental side divided by ^{3}H-activity on the control side. Abscissa: band number.

In two experiments, the ^{14}C- and ^{3}H-leucine were applied to the same eye 24 hr and 6 hr before sacrifice. No essential differences were observed between the two isotopes (fig. 1D). This suggests that the two different waves of transport observed within the first 24 hr [2, 3, 19] do not contain qualitatively different proteins.

In two experiments simultaneous injection of ^{3}H-leucine intravenously (200 μCi in 0.5 ml of Ringer solution) and of ^{14}C-leucine intraocularly was performed 24 hr before sacrifice. The difference in ^{14}C-activity between experimental and control side were computed and referred to the ^{3}H-activity. This ratio varied from one band to another (fig. 2A), leaving open the answer as to whether migrating synaptosomal proteins differ from those synthesized locally. In contrast, the ratio of ^{14}C- to ^{3}H-activity on the control side (fig. 2B) as well as of the ^{3}H-activity of both sides (fig. 2C) remained almost constant, thus supporting the reliability of the method.

In conclusion, by means of SDS polyacrylamide gel electrophoresis, the distribution pattern of radioactivity in the bands was different for those proteins migrating from the cell body with the fast axoplasmic flow, those migrating with the slow axoplasmic flow and those synthesized locally in the optic lobe and appearing in the synaptosomal fraction. However, a complete separation of the synaptosomal proteins as a function of their origin was not obtained.

Acknowledgements

Supported by grants no. 4806, 3.329.70, 3.133.69 and 3.137.69 of the Swiss National Foundation for Scientific Research. The expert technical assistance of Mrs. L. Heeb is gratefully acknowledged.

References

[1] B. Grafstein, Adv. Biochem. Psychopharmacol. 1 (1969) 11.
[2] M. Cuénod, J. Schonbach, J. Neurochem. 18 (1971) 809.
[3] J. Schonbach, M. Cuénod, Exp. Brain Res. 12 (1971) 275.
[4] J. Schonbach, Ch. Schonbach, M. Cuénod, J. Comp. Neurol. 141 (1971) 485.
[5] E. Mehl, in: Proc. Intern. Symp. on Macromolecules and the Function of the Neuron, Castle Liblice, 1967 (Excerpta Medica Foundation, Amsterdam) p. 22.
[6] C.W. Cotman, H.R. Mahler, Arch. Biochem. Biophys. 120 (1967) 384.
[7] C.W. Cotman, H.R. Mahler and R.E. Hugli, Arch. Biochem. Biophys. 126 (1968) 821.
[8] B. Bosmann, K.R. Case, M.B. Shea, FEBS Letters 11 (1970) 261.
[9] E.G. Gray and V.P. Whittaker, J. Anat. 96 (1962) 79.
[10] R. Lim and E. Tadayyon, Anal. Biochem. 34 (1970) 9.
[11] J.V. Maizel, Jr., D.F. Summers and M.D. Scharff, J. Cell Physiol. 76 (1970) 273.
[12] R.M. Grossfeld and E.M. Schooter, J. Neurochem. 18 (1971) in press.
[13] P.V. Tishler and Ch.J. Epstein, Anal. Biochem. 22 (1968) 89.

[14] A.L. Koch, Anal. Biochem. 23 (1968) 352.
[15] S. Bramhall, N. Noack, M. Wu and J.R. Loewenberg, Anal. Biochem. 31 (1969) 146.
[16] B.S. Mc Ewen and B. Grafstein, J. Cell Biol. 38 (1968) 494.
[17] J.O. Karlsson and J. Sjöstrand, J. Neurobiol. 2 (1971) 135.
[18] J. Boesch, P. Marko and M. Cuénod, in preparation.
[19] J.O. Karlsson and J. Sjöstrand, J. Neurochem. 18 (1971) 749.