Volume 263, number 1, 163–165 FEBS 08322 April 1990

Regional expression of a MCD-peptide and dendrotoxin I-sensitive voltage-dependent potassium channel in rat brain

Jean-Marc Séquier, John Brennand*, Jacques Barhanin and Michel Lazdunski

Institut de Pharmacologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique, UPR 411, 660 route des Lucioles, Sophia-Antipolis 06560 Valbonne, France and **ICI Pharmaceuticals, biotechnology Dept., Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK*

Received 30 January 1990; revised version received 19 February 1990

In situ hybridization histochemistry has been used to analyze the regional expression of a class of voltage-dependent K^+ channel that is sensitive to two polypeptide toxins (MCD peptide and dendrotoxin I) that produce spectacular effects on brain function. A heterogeneous expression of this K^+ channel was observed throughout the brain. High mRNA contents were observed in the granule cells of the gyrus dentatus as well as in pyramidal cells of the Ammon horn (CA3 > CA1) and in the cerebellum. Conversely, low levels of expression were found in basal ganglia (caudate putamen, globus pallidus, and ventral pallidum).

Hybridization, in situ; Hippocampus; Ionic channel

1. INTRODUCTION

Both the mast cell degranulating peptide (MCD) isolated from bee venom and dendrotoxin I (DTX_I) isolated from snake venom (i) are potent blockers of voltage-sensitive K^+ channels [1], (ii) induce neurotransmitter release and (iii) are potent convulsants since they induce limbic epilepsy when injected i.c.v. at high concentrations. At lower concentrations they induce hippocampal θ rhythm which is associated to arousal [2,3] and long-term potentiation (LTP) upon application of MCD to hippocampal slices [4]. The K^+ channel protein(s) containing binding sites for both DTX_I and MCD have been recently isolated and reconstituted [5,6].

A series of mammalian brain K^+ channels have been cloned recently [7–10]. These different isotypes of voltage-sensitive K^+ channels form, as previously described for *Drosophila* [11], a large family of proteins which differ by their chemical structures, their biophysical properties and their pharmacological profiles [12].

One of these rat brain cloned rat brain K^+ channels called RCK1 expresses both sensitivity to MCD and DTX_I [12]. The purpose of this work is to analyze the brain distribution of this K^+ channel sub-type using in situ hybridization histochemistry with RNA probes. This technique has been shown to be capable of differentiating the presence of mRNA populations having a certain degree of homology [13].

Correspondence address: M. Lazdunski, Institut de Pharmacologie Moléculaire et Cellulaire, UPR 411 du CNRS, 660 route des Lucioles, Sophia Antipolis 06560 Valbonne, France

2. MATERIALS AND METHODS

Wistar rats were injected with pentobarbital for intracardiac perfusion with an ice-cold PBS solution containing 4% paraformaldehyde. Brains were removed and postfixed in the same solution for 2 h and then kept overnight at 4°C under gentle agitation in a 20% saccharose/PBS solution before freezing. Twelve μm cryostat sections on 3-aminopropylethoxysilane coated slides were used and kept at −20°C. For hybridization, sections were brought to room temperature for 2 h and directly hybridized with 25 μl of buffer composed of 4 × SSC, 50% formamide, 2% Denhardt's solution, 1% *N*-lauroylsarcosine, 0.12 M phosphate buffer (pH 7.2), 10% dextran sulfate and 10 mM 2-mercaptoethanol containing the radioactive probe at a concentration of 3×10^7 dpm/ml. After an overnight hybridization at 60°C, the sections were washed in 1 × SSC, 10 mM 2-mercaptoethanol first at room temperature for 1 h and then at 75°C for 1.5 h. Sections were dehydrated in 0.03 M ammonium acetate/ethanol and exposed to β-max Hyperfilm (Amersham).

The K^+ channel cDNA probe used in this work is the mouse brain equivalent of RCK1 [12]. It was obtained with the polymerase chain reaction using primer sequences corresponding to the previously described mouse brain K1 potassium channel (MBK1) cDNA clone [10]. It differed from RCK1 at only 6 (over 495) amino-acid positions. This cDNA (called PCR3) was cloned into the *Sma*I site of bluescript KS (Stratagene) in both orientation in order to generate single-stranded RNA probes sense and anti-sense using the T7 polymerase (RNA transcription kit from Stratagene). Highly [^{35}S]thiotriphosphate labelled RNA probes were obtained following the Stratagene instructions and were purified and subjected to limited alkaline hydrolysis as described [13].

3. RESULTS AND DISCUSSION

Cells containing BK1 mRNA are found largely distributed in the brain (Fig. 1). A strong hybridization

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

signal is found in granule cells of the hippocampal formation as well as in the pyramidal cells of Ammon's horn (Fig. 1A). However, a striking difference is observed between pyramidal cells. CA3 cells seem to contain more BK1 mRNA than CA1 and CA2 cells (Fig. 1C).

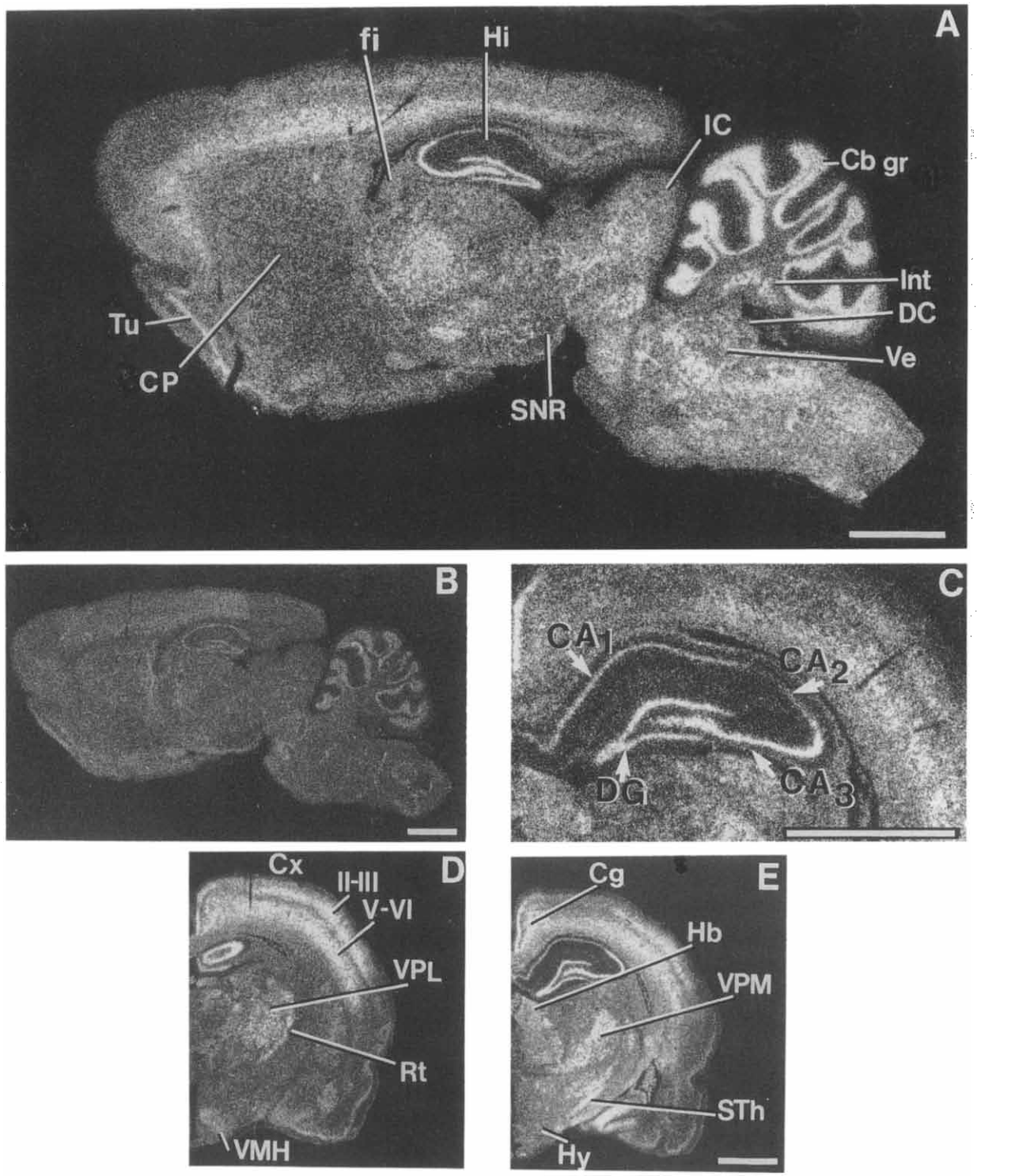

Fig. 1. (A) Parasagittal rat brain section hybridized with ^{35}S-labelled anti-sense single-stranded RNA probe complementary to PCR3. Cbgr, granule cell layer of the cerebellum; CP, caudate-putamen; DC, dorsal cochlear nucleus; fi, fimbria; Hi, hippocampus; IC, inferior colliculus; Int, nucleus interpositus. (B) Rat brain section hybridized with the control ^{35}S-labelled sense probe. (C) Distribution of BK1 mRNA in hippocampus and dentate gyrus (DG). Note the intense staining of CA3 cells compared to CA1 and CA2 cells. (D,E) Coronal sections of rat brain revealing several positive brain nuclei. Cg, cingulate cortex; Cx, cerebral cortex; Hb, habenular nucleus; Hy, hypothalamus; Rt, reticular thalamic nucleus; STh, subthalamic nucleus; VMH, ventromedial hypothalamic nucleus; VPL, ventral posterolateral thalamic nucleus; VPM, ventral posteromedial thalamic nucleus. Bar = 2 mm.

A high hybridization signal is observed in the cerebellum. The K^+ channel mRNA is expressed mainly at the level of the granule cell layer and in the Purkinje cells layer whereas the molecular layer appears devoid of autoradiographic grains. Highly labelled cells (Fig. 1A) are also observed in the nucleus interpositus which receives inputs from the Purkinje cells.

In the telencephalon, cells expressing BK1 in the cerebral cortex are distributed mainly in layers II, III and V, VI (Fig. 1D). A high hybridization signal has been found in the cingulate cortex (Fig. 1C) as well as in the piriform cortex and olfactory tubercule (Fig. 1A). In the basal ganglia, the hybridization signal has been found to be nearly absent for example in the caudate-putamen, the nucleus accumbens and in the pallidal regions (Fig. 1A) while few positive cells appear in the substantia nigra reticulata (Fig. 1A).

In the diencephalon, an autoradiographic signal is detected in the thalamus in particular in the ventrolateral and ventroposteromedial nuclei (Fig. 1A, D, E). The reticular thalamic nucleus and the subthalamic nucleus appear also positive (Fig. 1A) as well as the habenular nucleus (Fig. 1E). On the other hand, a low mRNA level has been found for the whole hypothalamus except for the ventromedial nucleus (Fig. 1D).

Cells containing high levels of BK1 mRNA are found in the brainstem for example in the midbrain at the level of the inferior colliculus and in the pons (Fig. 1A). Almost all nuclei of the hindbrain appear to be positive for BK1 mRNA like the vestibular and cochlear nuclei, the parvocellular reticular nucleus and trigeminal nucleus. All these hybridization signals reflect quite well the content of BK1 mRNA since a weak background signal is obtained when a control probe is used (Fig. 1B).

Results presented in this work reveal an expression pattern for the K^+ channel which is clearly different from that reported for another brain voltage sensitive K^+ channel clone called BK2 which is ubiquitously expressed in the brain [9]. One observes here a clearly heterogeneous expression since no BK1 encoding mRNA can be detected in the basal ganglia while adjacent regions like thalamic nuclei express it abundantly. In addition to these qualitative differences, quantitative ones were also observed for example among pyramidal cells all along the Ammon's horn.

There are clearly a variety of different K^+ channels of different sensitivities for DTX_1 and MCD in rat brain [12], most high affinity DTX_I binding sites are associated with relatively low affinity binding sites for MCD [3]. The voltage-sensitive K^+ channel that has been localized in this work has K_d values for the blockade by the different toxins of about 1–10 nM (DTX and DTX_I) and 50–100 nM (MCD) ([12], and Romey, G., personal communication). Long-term potentiation with MCD has also been observed after application to hippocampal slices of peptide concentrations of about 100 nM [4]. There are reasonable chances that the voltage-sensitive K^+ channel that has been mapped in this work and which is strongly represented in hippocampus is involved in hippocampal θ rhythm and LTP which was shown to be associated with a MCD effect in the CA1 region of hippocampus [4]. It is in the CA3 region of hippocampus that the epileptogenic properties of MCD have been identified [14].

Acknowledgements: This work was supported by grants from DRET (88/054) and Produits Roche SA. We would like to thank F. Aguila and C.R. Bettelheim for their very competent help.

REFERENCES

[1] Moczydlowski, E., Lucchesi, K. and Ranvindran, A. (1988) J. Membr. Biol. 105, 95–111.
[2] Bidard, J.-N., Gandolfo, G., Mourre, C., Gottesman, C. and Lazdunski, M. (1987) Brain Res. 418, 235–244.
[3] Bidard, J.-N., Mourre, C., Gandolfo, G., Schweitz, H., Widmann, C., Gottesmann, C. and Lazdunski, M. (1989) Brain Research 495, 45–57.
[4] Cherubini, E., Ben Ari, Y., Gho, M., Bidard, J.-N. and Lazdunski, M. (1987) Nature 328, 70–73.
[5] Rehm, H. and Lazdunski, M. (1988) Proc. Natl. Acad. Sci. USA 85, 4919–4923.
[6] Rehm, H., Pelzer, S., Cochet, C., Chambaz, E., Tempel, B.L., Trautwein, W., Pelzer, D. and Lazdunski, M. (1989) Biochemistry 28, 6455–6460.
[7] Baumann, A., Grupe, A., Ackermann, A. and Pongs, O. (1988) EMBO J. 7, 2457–2463.
[8] Christie, M.J., Adelman, J.P., Douglass, J. and North, R.A. (1989) Science 244, 221–224.
[9] McKinnon, D. (1989) J. Biol. Chem. 264, 8230–8236.
[10] Tempel, B.L., Jan, Y.N. and Jan, L.Y. (1988) Nature 332, 837–839.
[11] Tempel, B.L., Papazian, D.M., Schwarz, T.L., Jan, Y.N. and Jan, L.Y. (1987) Science 237, 770–775.
[12] Stühmer, W., Ruppersberg, J.P., Schröter, K.H., Sackmann, B., Stocker, M., Giese, K.P., Perschke, A., Baumann, A. and Pongs, O. (1989) EMBO J. 8, 3235–3244.
[13] Séquier, J.M., Richards, J.G., Malherbe, P., Price, G.W., Mathews, S. and Möhler, H. (1988) Proc. Natl. Acad. Sci. USA 85, 7815–7819.
[14] Cherubini, E., Neuman, R., Rovira, C. and Ben Ari, Y. (1988) Brain Res. 445, 91–100.