PROTEIN KINASE C ACTIVITY IN BRAIN TISSUE OF SPONTANEOUSLY HYPERTENSIVE RATS

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UDC 616.12-008.331.1-021.3-092.9-07: 616.831-008.931

KEY WORDS: spontaneous hypertension in rats; protein kinase C; synaptosomes

Activity of Ca^{++}, phospholipid-dependent protein kinase (protein kinase C; PKC) is increased in erythrocytes and platelets of spontaneously hypertensive (SHR) rats [15]. PKC is known to be involved in the regulation of neurotransmitter release from nerve cells [18]. It can be tentatively suggested that the higher level of secretion of transmitters from the brain synaptosomes of SHR rats compared with animals of the control group [8, 17] is connected with changes in this enzyme system in hypertension.

The aim of this investigation was to compare activity of PKC in the brain tissues of SHR and WKY rats of the control group.

EXPERIMENTAL METHOD

Male SHR (spontaneously hypertensive rats, Kyoto–Wistar strain) rats aged 14-15 weeks and weighing 180-220 g (BP 173 ± 5 mm Hg) and WKY rats (normotensive Kyoto–Wistar rats) of the control group, of the same sex and age (BP 120 ± 3 mm Hg) were used. The rats were decapitated and the brain without the cerebellum was homogenized in a Teflon–glass homogenizer (clearance 0.08-0.1 mm) in medium of the following composition (in mM): Tris-HCl 20 (pH 7.5), EGTA 5, EDTA 2, 2-mercaptoethanol 50, Triton X-100 0.1%, phenylmethylsulfonyl fluoride (PMSF) 0.65, leupeptin 0.01. The suspension was centrifuged at 105,000g for 50 min. The residue was discarded and the supernatant applied to a column with DEAE-cellulose (DE-52, from "Whatman," England), equilibrated with 20 mM HEPES-Tris (pH 7.5), 50 mM mercaptoethanol, and 1 mM EGTA. Protein was eluted with 0.2 M NaCl and determined by Lowry's method [10]. PKC activity was measured as incorporation of ^{32}P into histone H_1. In brief, to 150 µl of a sample containing 2-5 µg of isolated protein at 0-2°C, 50 µl of quadruple measuring medium (200 mM Tris-HCl, pH 7.5, 40 mM Mg-acetate, 8 mM $CaCl_2$, 50 µM ATP, and 0.25-0.5 µCi of γ-^{32}P-ATP) was added. The samples were incubated for 2-3 min at 30°C. PKC activity was measured in the presence of phosphatidylserine (10 µg per sample) and of 2.5×10^{-7} M phorbol ester (12,0-tetradecanoylphorbol-13 acetate, TPA). Basal activity was measured in the absence of the lipid and phorbol ester. The reaction was stopped by applying the sample to GF/F ("Whatman") filters, soaked beforehand with 1 ml of cold 25% TCA. The filters were then washed with 5 ml of cold 5% TCA. They were then put into flasks and radioactivity counted on a Delta-300 β-counter (USA), filled with Bray's scintillator.

EXPERIMENTAL RESULTS

Table 1 gives the results of measurement of activity of the basal kinases in the brain of SHR and WKY rats (the sum of activities of the different kinases, independent of the presence of phosphatidylserine and diacylglycerol) and of PKC. Basal kinase activity and PKC activity of the SHR rat brain were 20 and 35% higher respectively than in WKY rats. Total PKC activity (cytosol and membrane fractions) is shown because 0.1% Triton X-100, present in the isolation medium, solubilizes the membrane-bound enzyme. The properties of membrane-bound PKC do not differ from those of the cytosol form [7]. The increase in brain PKC activity of rats with hypertension may be due to higher activity of each enzyme molecule in SHR rats than in WKY rats, the number of PKC molecules in the two groups remaining unchanged, and to an increase in the concentration of PKC molecules in SHR rats compared with WKY rats, activity of each molecule in the two groups remaining the same, and also on account of both mechanisms.

Central Research Laboratory, Ministry of Health of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 7, pp. 42-44, July, 1989. Original article submitted September 20, 1988.

 0007-4888/89/0007-0952$12.50

TABLE 1. PKC Activity (in pmoles ^{32}P/min/mg protein) in Rat Brain Tissue (M ± m)

Group of animals	Number of animals	Basal activiy	PKC activity
WKY rats (control)	11	153,2±11,2	1864,7±156,3
SHR rats	10	189,3±11,6	2485,6±169,7
p		<0,05	<0,01

To solve the problem of the enzyme concentration in the brain of SHR and WKY rats, further investigations are needed to study the kinetics of binding of the labeled compounds, for which PKC would be the receptor (for example, with labeled phorbol esters).

In brain tissue, which is the richest in PKC [8], phosphoinositides are quickly metabolized, with the formation of diacylglycerol, an endogenous activator of PKC [6, 11], PKC, as a key enzyme of nerve tissue, is involved in regulation of the intracellular pH [13], membrane permeability for ions [6], and synaptic transmission [14]. Most of these processes are evidently controlled through phosphorylation by PKC of cytoskeletal components of nerve cells [12].

As was shown previously, the rate of entry of Ca^{++} along calcium channels into the brain synaptosomes is higher in SHR than in WKY rats [1]. Meanwhile, secretion and uptake of GABA and noradrenalin are changed in synaptosomes of SHR rat cells [4, 9]. Considering the regulating role of PKC in these processes and the results of this investigation it can be postulated that the mechanism of change of ion transport and transmitter secretion in hypertension is determined by enhanced PKC activity.

Enhanced PKC activity in the platelets of SHR rats [15] and erythrocytes of patients with essential hypertension [2] (but not with secondary forms of hypertension), in conjunction with the results of the present investigation, support the participation of this enzyme system in the pathogenetic mechanism of spread of membrane disturbances observed in primary hypertension in cells of different types [3].

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