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Distribution of Protein Kinase C in the Hippocampus of the Gerbil and Rat: Autoradiographic Analysis by [^{3}H]Phorbol 12,13-Dibutyrate

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Abstract—In-vitro quantitative receptor autoradiography with [^{3}H]phorbol 12,13-dibutyrate (PDBu) was used to determine the affinity constant (K_d) and the maximum number of receptor sites (B_{max}) for protein kinase C (PKC) in subregions of the gerbil hippocampus, and to compare the distribution of [^{3}H]PDBu binding sites in the gerbil hippocampus with that in the rat hippocampus. The K_d and B_{max} values in the subregions of the gerbil hippocampus were estimated at 2·6–3·8 nM and 2·38–2·54 pmol (mg tissue)$^{-1}$, respectively. The distribution of hippocampus [^{3}H]PDBu binding sites was uniform in the gerbil but not in the rat. The [^{3}H]PDBu binding activities in the strata oriens of the CA1 and CA3 subfields and the molecular layer of the dentate gyrus in the rat hippocampus were significantly higher than in the gerbil hippocampus. However, binding activity in the stratum lacunosum-moleculare of the rat CA1 subfield was statistically lower. These data demonstrate a difference in the distribution of [^{3}H]PDBu binding activity in the hippocampus between the gerbil and rat.

The Mongolian gerbil has several physiological and behavioural attributes that are analogous to similar phenomena in man, and that make them particularly useful as models of stroke, epilepsy, memory, auditory processes and several behavioural paradigms.

Protein kinase C (PKC), a calcium- and phospholipid-dependent enzyme, is highly concentrated in the rat (Kikkawa et al 1982) and is one of the key regulators of signal transmission (Miller 1986). This enzyme also plays a critical role in long-term potentiation (LTP) in the hippocampus (Akers et al 1986; Malenka et al 1986; Nishizuka 1986; Hu et al 1987), which is related to memory and learning (Lynch & Baudry 1984).

Hippocampal CA1 pyramidal cells in the gerbil and rat are selectively damaged by transient forebrain ischaemia (Kirino 1982; Pulsinelli et al 1982), and PKC may be important in the post-ischaemic modulation of neuronal activity and damage to CA1 pyramidal cells (Miller 1986; Louis et al 1988; Onodera et al 1989; Hara et al 1990b,c). In particular, in the gerbil, there is evidence that PKC activation is involved in post-ischaemia hippocampal CA1 pyramidal cell damage. PKC inhibitors such as K-252a (Yoshidomi et al 1989) and staurosporine (Hara et al 1990b) are reported to protect against CA1 pyramidal cell loss. However, the K_d and B_{max} values of phorbol 12,13-dibutyrate (PDBu) binding in subregions of the gerbil hippocampus have not been reported.

We reported a difference in sensitivity to the neuroprotective effect of a PKC inhibitor against post-ischaemic neuronal damage in the hippocampus between the gerbil and the rat (Hara et al 1990b). We therefore wished to examine the distribution of [^{3}H]PDBu binding activity in the hippocampus in the gerbil and the rat.

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Materials and Methods

Animal experiments

Male adult Mongolian gerbils (Seiwa Experimental Animal Ltd, Fukuoka, Japan, n = 8), 70–80 g, and Wistar rats (Japan SLC Ltd, Osaka, Japan, n = 4), 250–300 g, were used. After decapitation the brain was quickly removed, frozen in powdered dry ice, and stored at −80°C until assay. The brain was cut into 12 μm coronal sections in a cryostat and thaw-mounted onto gelatin-coated slides.

Autoradiography

[^{3}H]PDBu autoradiography was carried out as described by Worley et al (1986). The brain sections for the saturability test and for the estimation of K_d and B_{max} values in the subregions in the gerbil hippocampus were incubated with [^{3}H]PDBu (New England Nuclear, UK, sp. act. 13·2 Ci mmol^{-1}), over the range 0·5 to 50 nM, for 60 min at 25°C in 50 mM Tris-HCl buffer (pH 7·7) containing 100 mM NaCl and 1 mM $CaCl_2$. The sections used to compare distribution patterns of [^{3}H]PDBu binding were incubated with 2·5 nM [^{3}H]PDBu. Non-specific binding was assessed in the presence of 1 μM PDBu (Sigma, St Louis, MO, USA). After incubation and washing, the sections were dried under a stream of cold air. The dried slides were apposed to tritium-sensitive film (Amersham, Sweden, Hyperfilm-^{3}H) with tritium standards (Amersham [^{3}H]micro-scale) at room temperature (21°C) in X-ray exposure holders. After appropriate exposure times, the films were developed and fixed. The optical densities of the regions of interest were measured using an image analyser system (VIDAS Image Analyzer System, Zeiss, Germany). The relation between optical density and radioactivity was obtained with reference to the [^{3}H]micro-scale co-exposed with the sections, using a third-order polynomial function. Optical densities of the brain regions measured were within the range in which the optical

density and the tritium micro-scale radioactivity showed a near-linear relation. Specific binding was determined by subtracting the value for non-specific binding from that for total binding. Since there was no specific difference in non-specific binding, the difference in specific binding was probably not an artifact of the difference in the tritium quenching level (Onodera et al 1987a).

Statistics

The values obtained were expressed as means ± s.e.m. and statistical comparisons were made using the two-tailed Mann-Whitney U-test.

Results

K_d and B_{max} values in the gerbil hippocampus

The distribution of [^{3}H]PDBu binding activity in the whole gerbil brain was homogeneous and the hippocampal formation exhibited a high grain density, as did rat brain.

Specific and non-specific binding of [^{3}H]PDBu in the stratum oriens of the CA1 subfield, in which ischaemia-induced increase in the [^{3}H]PDBu binding activity was reported in the gerbil (Hara et al 1990c) and rat (Onodera et al 1989), are shown in Fig. 1. The specific binding in all hippocampal subregions was saturable as demonstrated in the stratum oriens of the CA1 subfield (Fig. 1). Scatchard analysis revealed K_d and B_{max} values of [^{3}H]PDBu binding in the CA1 subfield stratum oriens to be 2·9 nM and 2·5 pmol mg^{-1}, respectively (insert in Fig. 1). Subregional K_d and B_{max} values in the gerbil hippocampus were estimated at 2·6–3·8 nM and 2·4–2·5 pmol mg^{-1}, respectively, and are summarized in Table 1.

Comparative study of distribution in the gerbil and rat hippocampus

Fig. 2 shows the distribution of [^{3}H]PDBu binding activity in hippocampus of the gerbil (A) and rat (B), and Fig. 3 shows the subregional binding activities of [^{3}H]PDBu. The distribution of [^{3}H]PDBu binding activity in the hippocampus was rather uniform in the gerbil but not in the rat (Fig. 2). This was emphasized by subregional measurement of [^{3}H]PDBu binding activity (Fig. 3). Although subregions in the gerbil hippocampus had similar values (0·98–1·23 pmol mg^{-1}), in the rat the grain density in the stratum oriens of the CA3 subfield (1·64 ± 0·11 pmol mg^{-1}) was about twice that in the stratum lacunosum-moleculare of the CA1 subfield (0·84 ± 0·03 pmol mg^{-1}). Moreover [^{3}H]PDBu binding activity was significantly higher in most rat hippocampal subregions as compared with the gerbil, particularly in the strata oriens of the CA1 ($P<0·05$) and CA3 ($P<0·01$) subfields and in the molecular layer of the dentate gyrus

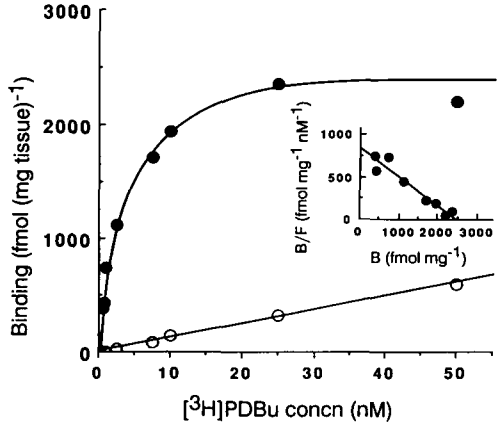

FIG. 1. Specific and non-specific binding of [^{3}H]phorbol 12,13-dibutyrate (PDBu) in the stratum oriens of the CA1 subfield in the gerbil hippocampus. Insert: Scatchard analysis of the specific binding. ○ Specific binding, ● non-specific binding.

Table 1. K_d and B_{max} values of [^{3}H]phorbol 12,13-dibutyrate binding in various hippocampal areas of gerbils.

Areas	K_d (nM)	B_{max} (pmol mg^{-1})
CA1 subfield		
Stratum oriens	2·9 ± 0·34	2·49 ± 0·18
Stratum radiatum	2·6 ± 0·22	2·44 ± 0·13
Stratum lacunosum-moleculare	3·8 ± 0·48	2·53 ± 0·19
CA3 subfield		
Stratum oriens	3·0 ± 0·36	2·54 ± 0·19
Stratum radiatum	3·3 ± 0·30	2·49 ± 0·14
Dentate gyrus		
Stratum moleculare	3·1 ± 0·45	2·38 ± 0·21

Optical densities were converted to pmol (mg tissue)$^{-1}$ using [^{3}H]micro-scale. Results are given as means ± s.e.m. n = 8.

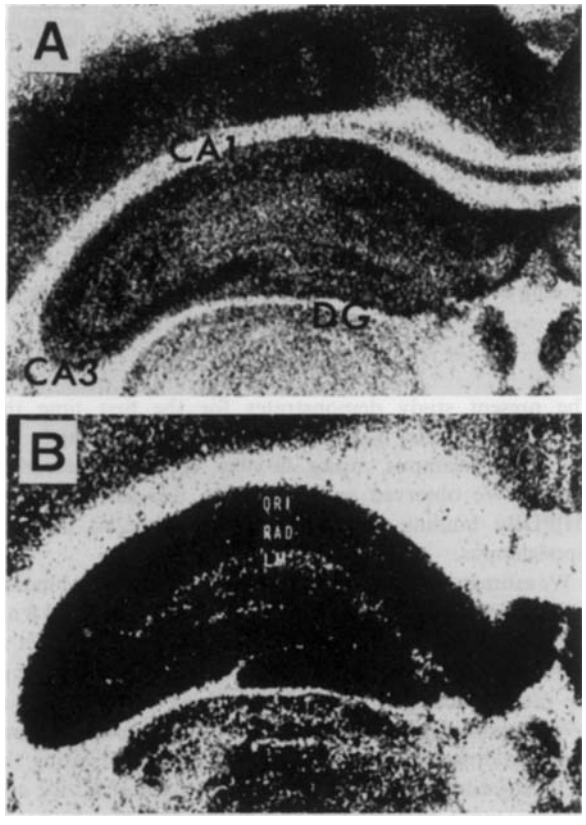

FIG. 2. Representative autoradiograms of hippocampus in the gerbil (A) and rat (B). ORI: stratum oriens, RAD: stratum radiatum, LM: stratum lacunosum-moleculare, DG: molecular layer of the dentate gyrus. Note the uniform distribution of [^{3}H]PDBu in the gerbil hippocampus as compared with that in the rat hippocampus.

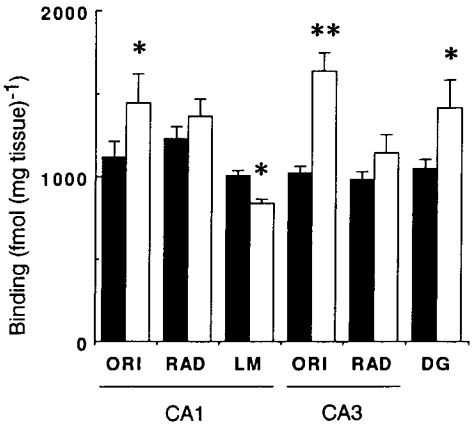

FIG. 3. Subregional binding activities of [^{3}H]PDBu (2·5 nM) in the gerbil and rat hippocampus. ORI: stratum oriens, RAD: stratum radiatum, LM: stratum lacunosum-moleculare, DG: molecular layer of the dentate gyrus. Values are means ± s.e.m. ■ Gerbil (n = 8), □ rat (n = 4). There are significant differences between the gerbil and rat, $*P < 0.05$, $**P < 0.01$.

($P < 0.05$). The stratum lacunosum-moleculare of the CA1 subfield was the only subfield in which [^{3}H]PDBu binding activity was higher in the gerbil than in the rat ($P < 0.05$). These results indicate that the distribution of [^{3}H]PDBu binding activity differs between gerbil and rat hippocampus.

Discussion

The present study demonstrates for the first time the subregional K_d and B_{max} values of [^{3}H]PDBu binding in the gerbil hippocampus, using in-vitro receptor autoradiography. We observed a difference in the distribution of [^{3}H]PDBu binding activity between the gerbil and rat hippocampus.

We estimated the K_d and B_{max} values of [^{3}H]PDBu binding in the subregions of the gerbil hippocampus to be 2·6–3·8 nM and 2·4–2·5 pmol (mg tissue)$^{-1}$, respectively. Battaini et al (1990) also found that the K_d value of [^{3}H]PDBu binding in the rat hippocampus was 2·8 nM. However, Niedel et al (1983) reported a K_d value of 7·0 nM in the homogenized rat brain. Additionally, Tanaka et al (1988), investigating the gerbil striatum, reported K_d and B_{max} values of 8·2 nM and 3·9 pmol mg^{-1}, respectively. This discrepancy may be due to a regional difference, as the K_d values of [^{3}H]PDBu binding varied among regions in rat brain (from 2·8 nM in the hippocampus to 19·9 nM in the cortex) (Battaini et al 1990).

Different distributions of subregional binding activity between the gerbil and rat hippocampus have been observed in [^{3}H]naloxone and [^{3}H]spiperone using in-vitro autoradiography (Onodera & Kogure 1988). On the other hand, the distribution of adenosine A_1, muscarinic cholinergic, $GABA_A$ and benzodiazepine binding sites in the gerbil hippocampus were similar to those in the rat (Onodera et al 1987a,b). We have also demonstrated that the distribution of [^{3}H]PDBu binding activity in the gerbil hippocampus differed from that in the rat. Thus, these differences between the gerbil and rat were observed not only in the signal transmission system but also in the signal transduction system, and may be related to the seizure-selective character of the gerbil. However, since we did not estimate K_d and B_{max} for PKC in the rat hippocampus in this study, the present experiment could not determine whether the observed differences in the binding distribution between the gerbil and rat hippocampus are due to differences in K_d or B_{max}.

In general, protective effects of drugs against post-ischaemic neuronal death have been greater in the gerbil ischaemia model than in the rat model. We recently found that the gerbil and rat have different sensitivities to staurosporine, a potent PKC inhibitor, and speculated that this may reflect a species difference (Hara et al 1990b). The species difference in the distribution of [^{3}H]PDBu binding activity may contribute to the difference in sensitivity to neuroprotective drugs. However, we cannot rule out the possibility that the differences in other mechanisms, such as excitatory amino acids (Benveniste et al 1984), free radical formation (Hara et al 1990a) and hypothermia (Busto et al 1987; Welsh et al 1990), play a role in the post-ischaemic neuronal death. In-vitro studies suggest the major part of non-activated PKC is located in the cytosolic fraction of the cell and tends to relocate to the membrane upon activation (Kaczmarek 1987). Thus, studies in the translocation of PKC activity in the gerbil and the rat are required.

In conclusion, we report subregional K_d and B_{max} values of [^{3}H]PDBu binding in the gerbil hippocampus, and a difference in the distribution of [^{3}H]PDBu binding activity between gerbil and rat hippocampus. Autoradiography using [^{3}H]PDBu in gerbil and rat may be expected to provide important information concerning the role of PKC in the brain under various physiological and pathological conditions after epilepsy and stroke and in memory storage processes.

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