**Impact of diabetes on CNS: Role of signal transduction cascade**

Sanjeev Kumar Bhardwaj, Sukhjit Kaur Sandhu, Poonam Sharma and Gurcharan Kaur*

*Department of Biotechnology, Guru Nanak Dev University, Amritsar, India*

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ABSTRACT: Diabetic neuropathy is the most common secondary complication of diabetes mellitus. Several pathogenetic factors have been proposed for diabetic neuropathy. The present investigation was undertaken to study different components of signal transduction from discrete brain regions from streptozotocin-induced diabetic rats. Rats were sacrificed after 1 and 3 months of induction of diabetes, and a control group was also studied in parallel to ascertain the specificity of diabetes-associated changes. Blood glucose level and protein content of discrete brain regions were also estimated. Signal transduction cascade components like protein kinase A, protein kinase C, cAMP, phospholipase C, phospholipase A$_2$, diacylglycerol and inositol phosphate levels were assayed in control and diabetic groups of rats. Significant attenuation in phosphoinositide metabolism along with activation of protein kinase activities were observed. These findings provide evidence to suggest a mechanism linking changes in signal transduction cascade, which is observed in 1- and 3-month diabetic rats, which ultimately leads to development of diabetic neuropathy. © 1999 Elsevier Science Inc.

KEY WORDS: Diabetic neuropathy, Signal transduction cascade, CNS, Protein kinases.

**INTRODUCTION**

Hyperglycemia induces a bewildering list of changes in neuronal and vascular cells in animal models of diabetes or diabetic patients. The pleotypic nature of the changes is not surprising, because the flow of glucose and its metabolites is known to affect many cellular pathways. A metabolically responsive, rapidly reversible slowing of nerve conduction in early diabetes in humans and experimental animals is thought to reflect underlying biochemical abnormalities in nervous tissue that may contribute to the development of diabetic neuropathy [4,32]. Several pathogenetic factors have been proposed for diabetic neuropathy. Among them, impairment of Na$^{+}$,K$^{+}$-ATPase activity in sciatic nerves has been claimed to play a pivotal role, supposedly related to derangement of phosphoinositide turnover, which in turn may result from altered myo-inositol uptake and metabolism [13,15]. Many previous reports have shown a decreased inositol metabolism in peripheral nerves from diabetic rats [12,29,31]. Slowing of nerve conduction has also been experimentally linked to activation of the polyol pathways by glucose and alterations in myo-inositol and phosphoinositol metabolism [14,39,46]. Another study by Zhu and Eichberg [48] reported that diacylglycerol (DAG) arises in large part from phosphoinositide metabolism in rat sciatic nerve from streptozotocin-induced diabetic rats.

Hermenegildo et al. [17] showed that the inhibition of protein kinase C (PKC) restores Na$^{+}$,K$^{+}$-ATPase activity in sciatic nerves of diabetic mice and suggested that enhanced PKC activity in diabetes phosphorylates ATPase enzyme and that the phosphorylated form of ATPase is less active. It is also known that γ-PKC (which is abundant in brain) activation can significantly potentiate another second messenger system called adenosine 3',5'-cyclic monophosphate (cAMP) [16]. Protein kinase C can phosphorylate Go involved in inhibition of adenylyl cyclase, thereby suppressing inhibitory input to cyclase and enhancing cAMP levels [21]. The aim of the present investigation is to study the effect of short- and long-term (4 and 12 weeks) diabetes on different components of signal transduction cascade such as protein kinase A (PKA), PKC, cAMP, phospholipase C (PLC), phospholipase A$_2$ (PLA$_2$), DAG and inositol 1,4,5-triphosphate (IP$_3$) and to find a correlation between diabetic neuropathy and signal transduction pathway.

**MATERIALS AND METHODS**

**Animals**

Thirty 3- to 4-month-old male Wistar rats were procured from a disease-free small-animal house, Haryana Agricultural University Hissar (India), and were housed in a group of three rats per cage in a temperature- (25 ± 2°C) and light-controlled (illuminated 5–19 h daily) environment with food and water provided ad libitum.

**Experimental Protocol**

All the procedures adopted for experiments were approved by the Council of Scientific and Industrial Research, India.

**Induction of Hyperglycemia**

To induce diabetes, rats were injected intraperitoneally with streptozotocin (Sigma, St. Louis, MO, USA) (50 mg/kg b.wt) in 0.1 M citrate buffer (pH 4.5, prepared fresh). Diabetes was confirmed by checking the urine sugar of these rats with diastics purchased from Autopak, Miles India Ltd., Mumbai. These ani-

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* Address for correspondence: Dr. Gurcharan Kaur, Department of Biotechnology, Guru Nanak Dev University, Amritsar-143 005 (Pb), India. Fax: (91)-183-258272; E-mail: bic_gdu@DBT.delhi.nic.in
mals were divided into two groups. One group of animals was sacrificed after 1 month of induction of diabetes, and the other group was maintained up to 3 months. Corresponding control animals received equivalent volume of buffer. Three animals were sacrificed in one experiment (one from each group: control, 1-month diabetic and 3-month diabetic), and each experiment was repeated at least five times. All the animals from these groups were sacrificed by decapitation after either 1 or 3 months of diabetes induction. The brains of all the animals were quickly removed and frozen in liquid nitrogen. Simultaneously, the blood was also collected to confirm the blood glucose level.

Preparation of Homogenates and Subcellular Fractions

Different brain areas such as the cerebral hemisphere, cerebellum, brain stem, thalamus and hypothalamus were microdissected. Ten-percent (w/v) homogenates of the different brain regions were prepared using a potter elvehjem-type homogenizer fitted with a Teflon® plunger. The homogenizing medium was composed of the components specific for the assay. The extracts were centrifuged (Biofuge 17RS, Heraeus Sepatech, Germany) at 12 000 × g for 40 min at 4°C as described earlier [22,23]. The supernatant fraction was separated from the pellet and was called a soluble fraction (SF). The pellet was washed with homogenizing medium once and resuspended in the same volume of the homogenizing medium and referred to as total particulate fraction (TPF).

Estimation of Protein Kinases and cAMP

Protein kinase A. Ten-percent homogenates were prepared in homogenizing medium containing 20 mM Tris/HCl (pH 7.4) containing 0.25 M sucrose and 0.1 mM dithiothreitol. The PKA activity was estimated from both the fractions as per the method of Roskoski [37]. One unit of the enzyme activity is defined as one μmole of NAD$^+$ formed/min/g tissue at 25°C.

Protein kinase C. The PKC activity was estimated according to the method of Wilkinson and Hallam [44]. One unit of enzyme activity is defined as the amount of enzyme required to phosphorylate 1 μmole of peptide/min/g tissue at 30°C.

cAMP. To measure the cAMP content, the samples were homogenized (10% w/v) in ice-cold 10% Trichloroacetic acid (TCA) followed by centrifugation at 3000 × g for 15 min at 4°C. After extraction with diethyl ether, samples were mixed with 1/20th volume of 0.25 M ZnSO$_4$ and Ba(OH)$_2$. The resulting precipitate of barium sulphate was removed by centrifugation at 4000 × g for 15 min at 4°C to eliminate any nucleotide such as Adenosine triphosphate (ATP), Adenosine diphosphate (ADP), 5’-AMP and 3’-AMP. The supernatants having cAMP were neutralized (pH 7.0–8.0) by titrating with NaHCO$_3$. The assay of cAMP based on the activation of glycogen phosphorylase was performed as per the method of Kakiuchi and Rall [20], which is a modified method of Butcher et al. [3]. The cAMP concentration was calculated as

![FIG. 1. Percent specific activity of protein kinase A from (A) soluble fraction and (B) total particulate fraction from the cerebral hemisphere (CH), cerebellum (CB), brain stem (BS), thalamus (TH) and hypothalamus (HT) after 1, D(1), and 3 months, D(2), of diabetes induction. Absolute specific activity (μmol/min/mg protein) was 0.044 ± 0.001, 0.045 ± 0.001, 0.044 ± 0.001, 0.039 ± 0.004, 0.046 ± 0.002 from soluble and 0.114 ± 0.07, 0.171 ± 0.008, 0.141 ± 0.014, 0.133 ± 0.009, 0.142 ± 0.008 from total particulate fraction from CH, CB, BS, TH and HT, respectively. Three animals were sacrificed in one experiment, and each experiment was repeated at least five times. Bars represent mean ± SEM. *p < 0.05; **p < 0.02; ***p < 0.01; ****p < 0.001.](image-url)
pmoles of cAMP/mg protein at 37°C taking purified cAMP as standard.

Estimation of Phospholipases, DAG and IP₃

**Phospholipase C.** For PLC assay, 10% w/v homogenates were made in homogenizing medium containing 20 mM Tris/HCl (pH 7.4), 0.25 M sucrose, 0.1 mM dithiothreitol, 10 mM EGTA, 2 mM EDTA and 1 mM PMSF. The PLC activity was assayed in both the subcellular fractions according to the method of Shimohama et al. [38]. One unit of enzyme activity is defined as 1 pmole of substrate utilized/min/g tissue at 37°C.

**Phospholipase A₂.** For PLA₂ assay, the samples were homogenized in a medium containing 20 mM Tris/HCl (pH 7.4), 0.25 M sucrose and 0.1 mM dithiothreitol. The PLA₂ was estimated in both fractions using chromogenic substrate (4-nitro-3-octanoyloxy) according to the method of Cho and Kezdy [7]. One unit of enzyme activity is defined as 1 µmole of substrate hydrolyzed/min/g tissue at 37°C.

**Diacylglycerol.** The DAG concentration was measured by preparing the 10% w/v homogenates in balanced salt solution containing 3.64 g NaCl, 0.2 g KCl, 0.68 g NaHCO₃, 0.1 g MgSO₄, 0.5 g glucose, 3.6 g HEPES, 0.08 g NaH₂PO₄ and 0.14 g CaCl₂ (pH 7.4) in total volume of 500 ml. Emulsion was prepared by mixing equal proportions of homogenates with ice-cold chloroform: methanol (1:1), followed by centrifugation at 2000× g for 2 min. The lower organic phase was dried in a vacuum after discarding the upper aqueous phase. Dried samples were solubilized by adding a 20-µl solution containing 7.5% (w/v) n-octyl-β-glucopyranoside and 5 mM cardiolipin in 1 mM DETAPAC, followed by sonicating water bath. The DAG estimation in these samples was performed according to the method of Kennerly et al. [23] as modified by Preiss et al. [34]. The amount of DAG present in the sample was calculated from the amount of [³²P]-phosphatidic acid produced and is expressed as pmoles [³²P]-phosphatidic acid produced/min/g tissue at 25°C.

**Inositol 1,4,5-triphosphate.** For measuring the IP₃ contents, 5% (w/v) homogenates were made in 15% (v/v) ice-cold TCA, followed by centrifugation at 2000× g for 15 min at 4°C. The supernatant were extracted three times with 10 volumes of water-saturated diethyl ether, and then the samples were neutralized to pH 7.5 by titrating with NaHCO₃. The IP₃ concentration was measured according to the method of Challiss [6] and expressed as pmoles of [³H]-IP₃ bound/min/g tissue at 4°C.

**Blood Glucose and Protein Assay**

Serum glucose from all the rats was estimated using a GOD/POD-based kit obtained from Autopak (Miles India Ltd.) and expressed in mg/dl. Protein content (mg/g tissue) of both the fractions was determined using the method of Lowry et al. [28].

**Statistical Analysis**

Values are expressed as mean ± SEM. The data were initially analyzed by one-way analysis of variance (ANOVA).
Acid produced/g tissue/min at 25°C. Inositol 1,4,5-triphosphate content is expressed as pmoles of [3H]-IP₃ bound/min/g tissue at 4°C. Each value is mean ± SEM of five separate experiments with three rats in each group.

Groups showed about a 50% increase over control levels of the enzyme activity, followed by hypothalamus PKC activity (p < 0.05) regions from the 3-month diabetic group. Both cerebral hemisphere and cerebellum also showed a significant increase from discrete brain regions, with much significant change after induction of diabetes. Only in the hypothalamus region was the PKC activity reduced to 22–25% as compared to control values in both the diabetic groups, whereas enzyme activity decreased to 18% in the cerebral hemispheres region from the 1-month diabetic group.

Diacylglycerol and IP₃ contents. Diabetic rats showed a marked decrease in DAG and IP₃ contents from all the brain regions studied. The decrease observed in the DAG content was statistically significant in both the 1- and 3-month diabetic groups, whereas the percentage of change was more pronounced in the 3-month diabetic group. The IP₃ content was also seen to decline in the 3-month diabetic group (from 29 to 62% of the control values), whereas the 1-month diabetic group showed only a marginal decline in IP₃ content, which was not statistically significant. Results are presented in Table 1.

**RESULTS**

**Blood Glucose and Protein Content**

Blood glucose levels (mM) were 18 mM and 23 mM after 1 and 3 months of diabetic period, respectively, as compared to control values of 7 mM. There was no statistically significant change in protein content of tissue in any of the three groups of animals.

**Effect of Diabetes on Protein Kinases and cAMP Content**

**Protein kinase A.** Induction of diabetes resulted in an increase in specific activity of PKA, with more pronounced changes observed in the 1-month diabetic group. The PKA activity in SF from the hypothalamus and brain-stem regions showed a maximum increase up to 67% and 50% (as compared to control values). In the 1-month diabetic group, enzyme activity associated with TPF also showed a significant increase from discrete brain regions, with thalamus showing a 77% increase over the control level. The percentage of increase in the 3-month diabetic group was less as compared to the 1-month diabetic group, and the brain stem, thalamus and hypothalamus in TPF showed a statistically significant change. Results are presented in Fig. 1.

**Protein kinase C.** The percent changes in the specific activity of PKC are shown in Fig. 2. The PKC activity was increased in the cerebral hemisphere, cerebellum and hypothalamus in both diabetic groups, but more significant change was observed in the 3-month diabetic group. Both cerebral hemisphere and cerebellum showed about a 50% increase over control levels of the enzyme activity, followed by hypothalamus PKC activity (~30% increase) in the 3-month diabetic group.

**cAMP.** The present results show a significant increase in cAMP level (pmole/mg protein) in the 3-month diabetic group. Brain-stem and hypothalamus regions showed a maximum percentage increase of 63% and 80%, respectively, as compared to the control group. In the 1-month diabetic group, only brain stem showed a significant increase. Results are presented in Table 1.

**Effect of Diabetes on Phosphoinositide Metabolism Components**

**Phospholipase C.** Figure 3 shows the comparison of specific activities of PLC in discrete brain regions in SF and TPF from the control and 3-month diabetic groups of animals. Different brain regions showed a decline of approximately 20 to 45% in PLC activity in SF (as compared to control values) in the 1-month diabetic group. The percentage decrease of PLC activity from SF in the 3-month diabetic group ranged from 15 to 50% from discrete brain regions. On the other hand, PLC activity in TPF did not show much significant change after induction of diabetes. Only in the hypothalamus region was the PLC activity reduced to 22–25% as compared to control values in both the diabetic groups, whereas enzyme activity decreased to 18% in the cerebral hemispheres region from the 1-month diabetic group.

**Diacylglycerol and IP₃ contents.** Diabetic rats showed a marked decrease in DAG and IP₃ contents from all the brain regions studied. The decrease observed in the DAG content was statistically significant in both the 1- and 3-month diabetic groups, but the percentage of change was more pronounced in the 3-month group. The IP₃ content was also seen to decline in the 3-month diabetic group (from 29 to 62% of the control values), whereas the 1-month diabetic group showed only a marginal decline in IP₃ content, which was not statistically significant. Results are presented in Table 1.

**Phospholipase A₂.** Figure 4 shows the percentage of decrease in specific activity of PLA₂ in SF and TPF from discrete brain regions of the control and diabetic groups of rats. Soluble-fraction-associated enzyme activity showed a more pronounced decrease as compared to the particulate fraction. In the 1-month diabetic group, the cerebral hemispheres and cerebellum showed maximum decline in PLA₂ activity of about 25% (below control value), whereas the percentage of decrease in the 3-month diabetic group ranged between 15 and 40% of the control values from all the brain regions studied. The PLA₂ activity in the particulate fraction declined only in the cerebral hemispheres (p < 0.02), brain stem (p < 0.02) and hypothalamus (p < 0.05) regions from the 3-month diabetic group.

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**TABLE 1**

**EFFECT OF SHORT- AND LONG-TERM DIABETES ON ADENOSINE-3’-5’ CYCLIC MONOPHOSPHATE (cAMP), DIACYLGLYCEROL (DAG) AND INOSITOL-1,4,5-TRIPHOSPHATE (IP₃) CONTENT FROM DIFFERENT REGIONS OF RAT BRAIN**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>CH</th>
<th>CB</th>
<th>BS</th>
<th>TH</th>
<th>HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAMP</td>
<td></td>
<td>0.209 ± 0.009</td>
<td>0.191 ± 0.009</td>
<td>0.141 ± 0.007</td>
<td>0.207 ± 0.007</td>
<td>0.132 ± 0.006</td>
</tr>
<tr>
<td>D (1)</td>
<td></td>
<td>0.225 ± 0.011</td>
<td>0.226 ± 0.013</td>
<td>0.198 ± 0.002</td>
<td>0.234 ± 0.003</td>
<td>0.153 ± 0.007</td>
</tr>
<tr>
<td>D (2)</td>
<td></td>
<td>0.251 ± 0.009</td>
<td>0.251 ± 0.011</td>
<td>0.230 ± 0.006</td>
<td>0.238 ± 0.005</td>
<td>0.238 ± 0.013</td>
</tr>
<tr>
<td>DAG</td>
<td></td>
<td>34.89 ± 1.74</td>
<td>34.22 ± 0.60</td>
<td>36.54 ± 1.25</td>
<td>33.17 ± 0.19</td>
<td>31.41 ± 1.92</td>
</tr>
<tr>
<td>D (1)</td>
<td></td>
<td>26.30 ± 0.29</td>
<td>26.52 ± 0.73</td>
<td>26.51 ± 0.47</td>
<td>26.21 ± 0.57</td>
<td>18.67 ± 0.39</td>
</tr>
<tr>
<td>D (2)</td>
<td></td>
<td>13.15 ± 1.47</td>
<td>18.35 ± 1.46</td>
<td>19.44 ± 0.63</td>
<td>11.62 ± 0.92</td>
<td>8.93 ± 0.44</td>
</tr>
<tr>
<td>IP₃</td>
<td></td>
<td>595.15 ± 6.58</td>
<td>764.92 ± 73.05</td>
<td>799.04 ± 62.85</td>
<td>699.95 ± 23.57</td>
<td>642.97 ± 49.10</td>
</tr>
<tr>
<td>D (1)</td>
<td></td>
<td>540.05 ± 59.01</td>
<td>607.95 ± 33.42</td>
<td>629.90 ± 83.02</td>
<td>492.93 ± 47.14</td>
<td>518.01 ± 44.99</td>
</tr>
<tr>
<td>D (2)</td>
<td></td>
<td>336.01 ± 25.05</td>
<td>314.11 ± 04.40</td>
<td>573.11 ± 23.80</td>
<td>266.60 ± 54.43</td>
<td>286.56 ± 11.90</td>
</tr>
</tbody>
</table>

Adenosine 3’-5’ monophosphate content is expressed as pmol/g protein at 37°C. Diacylglycerol content is expressed as nmoles of [3H]-phosphatidic acid produced/g tissue/min at 25°C. Inositol 1,4,5-triphosphate content is expressed as pmol/g protein at 4°C. Each value is mean ± SEM of five separate experiments with three rats in each group.

CH, cerebral hemisphere; CB, cerebellum; BS, brain stem; TH, thalamus; HT, hypothalamus; D (1) and (2), 1-month and 3-month diabetic duration groups.

Fischer’s p value: a p < 0.05; b p < 0.02; c p < 0.01; d p < 0.001.
DISCUSSION

Diabetic neuropathy is a common secondary complication of diabetes mellitus. Animal models of diabetes, including the streptozotocin-induced diabetic rats [42], have proved very useful in trying to determine the underlying cause of diabetic neuropathy. Some of the abnormalities demonstrated in experimental diabetic neuropathy include a decreased axonal transport, a reduced nerve conduction velocity, an increase in resistance to ischemic conduction failure and an impaired axon regeneration [2,4,26,30]. One main question facing investigators in this area is whether these diverse secondary complications associated with diabetic neuropathy are a result of the alterations in nervous system signal transduction cascade. The results of the present study in 1- and 3-month streptozotocin diabetic rat brain tissue reveal significant alterations in various components of signal transduction, namely PKA, PKC, cAMP, PLC, PLA2, IP3 and DAG.

Protein kinase C activity has been shown to increase in several tissues such as aorta, liver, heart and kidney from diabetic animals, which suggests a possible role of PKC in the development of diabetic complications [18,36,40]. However, there is no common consensus as to the direction of change in PKC activity in neuronal tissue, and this is highlighted by the apparently contradictory results obtained in sciatic nerves by different workers [17,36]. Hyperglycemia-associated modulation of PKC activity, in turn, mediates the adverse effects of diabetes through multiple pathways such as alteration of enzymatic activities like PLA2 and Na+,K+-ATPase and gene expression [19,24]. Impairment of Na+,K+-ATPase activity has been claimed to play a pivotal role in the pathogenesis of diabetic neuropathy. The Na+,K+-ATPase is phosphorylated by PKC, and the activators of PKC have been shown to inhibit Na+,K+-ATPase activity [1,27]. Moreover, inhibition of PKC has been shown to restore the decrease of Na+,K+-ATPase activity in the sciatic nerve of alloxan-induced diabetic mice [17].

Activation of PKC can induce significant changes, either facilitatory or inhibitory in cAMP accumulation [16]. Such interactions represent an example of “cross talk” between second messenger systems. The present results showed a significant increase in cytosolic PKC activity, with a greater percentage increase in the enzyme activity from the 3-month diabetic group. In the initial experimentation, PKC activity was also assayed in the TPF from discrete brain regions, which showed very little activity change, so in the experiments, only cytosolic PKC was studied in the 1- and 3-month diabetic groups. Protein kinase C activation was also associated with an increase in the cAMP/PKA system, and such cross talk between cAMP/PKA cascade and PKC has also been suggested in sensory neurons of aplysia, and many other cellular systems have been suggested [9,33]. One locus of interaction is the

FIG. 3. Percent specific activity of phospholipase C from (A) soluble fraction and (B) total particulate fraction from the cerebral hemisphere (CH), cerebellum (CB), brain stem (BS), thalamus (TH) and hypothalamus (HT) after 1, D(1), and 3 months, D(2), of diabetes induction. Absolute specific activity (pmoles/min/mg protein) was 2.57 ± 0.148, 1.65 ± 0.088, 2.32 ± 0.053, 2.44 ± 0.254, 2.34 ± 0.122 from soluble and 1.34 ± 0.032, 1.44 ± 0.008, 0.855 ± 0.051, 1.27 ± 0.035, 1.34 ± 0.060 from total particulate fraction from CH, CB, BS, TH and HT, respectively. Three animals were sacrificed in one experiment, and each experiment was repeated at least five times. Bars represent mean ± SEM. *p < 0.05; **p < 0.02; ***p < 0.01; ****p < 0.001.
G-protein (Gs)-sensitive adenylyl cyclase [9], and also at the level of receptor [35,47]. Decline in Na\(^{+}\),K\(^{+}\)-ATPase activity in diabetic neuropathy has also been related to derangement of phosphoinositide turnover, which in turn may result from the altered myo-inositol uptake and metabolism [13,15]. Phosphoinositides are involved in the control of membrane fluidity during axonal conduction, and alterations in their relative amounts or turnover rates may be related to the physiological changes of early diabetic neuropathy. Enzymes of phosphoinositol synthesis such as CDP-diacylglycerol-inositol phosphatidyltransferase, phosphatidylinositol kinase and phosphatidylinositol 4-phosphate kinase showed decreased specific activities in brain and sciatic nerves of diabetic rats [43]. These previous studies may explain the decline in IP3 and DAG content observed in the present investigation in the 1- and 3-month diabetic rat brain. Decreased enzyme activities of phosphoinositol metabolism may be pertinent to diabetic neuropathy because phosphatidylinositol and its derivatives are implicated in nerve conduction.

Zhu and Eichberg [48] determined the content of DAG in sciatic nerves from normal and streptozotocin-induced diabetic rats. Comparing the DAG molecular species distribution in desheathed normal and diabetic nerves, these authors observed a marked decrease in 18:0/20:4 DAG species in diabetic rats as compared to controls, which is consistent with its production from phosphoinositide. The connection between reduced phosphoinositides and decreased Na\(^{+}\),K\(^{+}\)-ATPase activity could be due to a fall in the DAG content, which is a natural activator of PKC. The DAG in turn is generated by PLC-mediated breakdown of phosphoinositides, which has also been shown to decrease in diabetic brain tissue along with DAG in the present investigation. The PLC activity decline in the brain is expected to affect mainly the 18:0/20:4 molecular species of DAG because this is the principal molecular species of phosphoinositides in the nervous tissue [43].

FIG. 4. Percent specific activity of phospholipase A\(_2\) from (A) soluble fraction and (B) total particulate fraction from the cerebral hemisphere (CH), cerebellum (CB), brain stem (BS), thalamus (TH) and hypothalamus (HT) after 1, D(1), and 3 months, D(2), of diabetes induction. Absolute specific activity (\(\mu\)moles/min/mg protein) was 0.107 ± 0.006, 0.133 ± 0.006, 0.119 ± 0.002, 0.140 ± 0.017, 0.090 ± 0.006 from soluble and 0.170 ± 0.003, 0.157 ± 0.001, 0.108 ± 0.011, 0.141 ± 0.007, 0.099 ± 0.001 from total particulate fraction from CH, CB, BS, TH and HT, respectively. Three animals were sacrificed in one experiment, and each experiment was repeated at least five times. Bars represent mean ± SEM. *\(p < 0.05\); †\(p < 0.02\); ‡\(p < 0.01\); §\(p < 0.001\).
of arachidonic acid in nervous tissue. Under normal conditions, PLA₂ is involved in maintaining normal cellular function by providing arachidonic acid eicosanoids [45]. Arachidonic acid modulates ion channels and regulates the activities of many enzymes such as PKA, DAG kinase, GTPase-activating proteins and also regulation of gene expression. It also inhibits glutamate uptake that is mediated by excitatory amino acid transport in intact cells, tissue slices and in various types of cell cultures of neuronal and glial origin [11]. In light of the previous reports illustrating the importance of signal transduction cascade in cell function, the present results of decline in DAG, IP₃, PLC and PLA₂ along with activation of cAMP/PKA and PKC suggest a direct correlation between diabetic neuropathy and signal transduction pathway in rat brain.

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