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Decreased Phosphorylation of GAP-43/B-50 in Striatal Synaptic Plasma Membranes after Circling Motor Activity

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The effects of spontaneous circling motor activity on the in vitro phosphorylation of the protein kinase C substrate GAP-43/B-50 was studied on striatal membranes of developing rats (30 days of age). At this time of postnatal development, permanent plastic changes in cholinergic and dopaminergic systems are produced by physiological motor activity. Exercised animals showed a significant reduction of 31% in the level of GAP-43/B-50 endogenous phosphorylation in the contralateral striatum respect to the ipsilateral side ($P < 0.01$), while control animals did not show asymmetric differences. Compared to controls, the contralateral striatum of exercised animals showed a 33% reduction in the incorporation of ^{32}P-phosphate into GAP-43/B-50 30 minutes post-exercise ($P < 0.01$). This change in GAP-43/B-50 phosphorylation was correlated with the running speed developed by the animals (r:0.8986, $P = 0.015$). GAP-43/B-50 immunoblots revealed no changes in the amount of this protein in any group. Moreover, a significant variation of 25% ($P < 0.05$) in the PKC activity was seen between both exercised striata. Interhemispheric differences were not found in control animals. We conclude that endogenous phosphorylation of this protein is also altered by motor activity in the same period that permanent changes in striatal neuroreceptors are triggered after motor training.

KEY WORDS: GAP-43/B-50; PKC; SPM; rat striatum; circling motor activity and development.

INTRODUCTION

The striatum, one of the principal components of the basal ganglia, exerts a main role in motor behavior by means of a highly functional organization (1). As a representative brain area of motor function, the striatum has been well characterized in terms of input-output connections, cytoarchitectural organization, neurotransmitter pathways and second messenger systems involved in normal activity (2,3).

Since striatal function impairment was related to several motor disturbances, the striatum has been investigated to understand the neurochemical basis underlying normal and pathological motor behavior.

Motor activity has been shown to affect several neurotransmitter systems (4–6) and neurotrophins (7,8), but it remains to be shown whether it can influence other key molecular systems which serve to the maintenance and plasticity of the brain. Although transient neurochemical modifications are normal events regarding neuronal activity during exercise, long term modifications have also been described in relation to motor activity in the striatum, only when the animals were trained at 30 days of age (9). This long-lasting variation could shed light in various aspects of motor behavior and motor plasticity. We have recently described a permanent coalteration of the muscarinic and dopaminergic striatal sys-

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tem after the performance of a circling motor training (10). Likely, other molecular mechanisms involved in neuronal activity and plasticity should be activated during motor stimulation in order to trigger these long term alterations in this particular period. As the phosphorylation processes play a key role in synaptic plasticity, we have focused on the phosphorylation of the growth-associated protein GAP-43 mediated by the protein kinase C (PKC).

GAP-43, also called B-50, pp46, F1 and Neuromodulin, localized in axons, growth cones and presynaptic nerve endings, is a calmodulin-binding protein substrate of PKC (11,12). This protein has been implicated in neuronal development, neurotransmission, and long term potentiation (13,14). It was suggested to be involved in morphological and functional neuroplasticity by way of its participation in plasma membrane signal transduction and neurotransmitter release (15,16).

It has been also described that amphetamine (AMPH) treatment results in a sensitization of locomotor behavior that is accompanied by an enhancement of PKC mediated-GAP-43 phosphorylation state in rat striatum (17). Moreover, the GAP-43 phosphorylation was correlated with locomotor activity during imprinting training in the left intermediate and medial hyperestriatum ventrale (IMHV) of chick (18).

In the present report we evaluate the PKC-dependent phosphorylation of GAP-43 after circling motor activity in the period where long term neuroreceptor changes are triggered, in order to investigate the possible participation of this phosphoprotein in the initial mechanisms that underlie motor plasticity.

EXPERIMENTAL PROCEDURE

Circling Motor Activity. Male Sprague-Dawley rats of 30 days of age were placed to run voluntarily over a giratory platform turning at 15 rpm. Each rat was required to perform 150 complete turns during the session of exercise (150 meters). The time was recorded in order to calculate the running speed. The animals with similar motor performance (speed) were grouped together for correlation studies. The direction of turn was randomly selected in each individual experiment and all the exercised animals turned in that direction. Control animals were placed in the arrested giratory platform and freely explored the novel environment. We have never recorded more than 10 turns per session for any control animal. Running behavior performed by exercised animals was not observed in controls.

Synaptic Plasma Membranes (SPM) Preparation. The experiments were performed 6 times using 3 to 5 animals for each experimental group. Left vs. right striata were grouped as ipsi vs. contralateral according to the turning direction (clockwise or anticlockwise) of its respective exercised group. Rats were killed by decapitation at 0 or 30 minutes post-exercise. Brains were quickly removed, left and right striata were dissected separately on ice and weighed. Tissue was added to ice cold 20 mM Tris-HCl buffer pH 7.4 (10%w/v solution), containing 0.32 M sucrose, 1 mM EDTA, 1 mM EGTA, 1 mM PMSF, 10 μM pepstatin and 10 μM leupeptin. Synaptic plasma membrane preparations were obtained according to a modification of the method described by Azcurra and De Robertis (19). To inhibit GAP-43/B-50 dephosphorylation SPM were prepared in the presence of EGTA as described Gnegy et al (17).

Tissue homogenization was performed at 1,300 rpm by 40 strokes in a potter-Elvehjem glass homogenizer fitted with a loose teflon pestle (clearence 0.25 mm). Following centrifugation at 1,090 *g* for 10 min, the pellets were washed twice and then discarded. Supernatants were centrifuged again at 13,000 *g* for 20 min (mitochondrial pellet). Following two washes, the pellets were resuspended in bidestilled water pH 6.8 (osmotic shock) and centrifuged at 20,200 *g* for 30 min. Then, pellets were resuspended in homogeneization buffer, loaded on a discontinuous sucrose gradient (0.8 to 1.0 M) and centrifuged at 100,000 *g* max for 80 min at 4°C in a Beckman 90 Ti rotor. The material floating on the 1 M band was collected, washed in same buffer, pelleted by centrifugation at 150,000 *g* for 80 min and finally resuspended in Buffer A (10 mM Tris-HCl buffer pH 7.4, 10 mM $MgCl_2$, 1 mM $CaCl_2$). Protein concentration was determined by the method of Lowry et al, using bovine serum albumin as standard (20).

GAP-43/B-50 Phosphorylation Assays. In vitro endogenous phosphorylation was performed in conditions known to activate PKC (21). Briefly, aliquots of SPM (10 μg) were preincubated for 5 min at 30°C in a final volume of 25 μl in buffer 10 mM Tris-HCl pH 7.4, 10 mM $MgCl_2$ and 1 mM $CaCl_2$. The reactions were initiated by the addition of 7.5 μM ATP and 2 μCi [γ-^{32}P]ATP (Dupont 6,000 Ci/mmol) in buffer A and terminated 1 min later by addition of 12.5 μl stopping solution (187.5 mM Tris-HCl pH 6.5, 6% SDS, 15% β-Mercaptoethanol, 30% glycerol and 0.003% Bromophenol blue). In some experiments, PKC inhibitors (H-7 and Staurosporine), phorbol ester (TPA), heparin or protein phosphatase inhibitors (okadaic acid and cypermetrin) were added to the incubation medium.

Electrophoresis. Samples containing 10 to 20 μg of SPM protein were subjected to a one-dimensional 12% SDS-polyacrilamide gel electrophoresis (SDS-PAGE). The gels were subsequently stained with Coomasie blue, destained and autoradiographied by exposing the gels to an AGFA-Curix film. Finally, densitometric analysis of the X-ray film was performed using a MCID Image Analysis System (5.02v, Image Research Inc., Ontario, Canada).

Western Blotting of GAP-43/B-50. SPM proteins (20 μg) were electrophoretically transferred (12 hs at 250 mA) to nitrocellulose membranes (pore size 0.45 μm) as described (22). After blocking, the membrane was incubated for 12 hs at room temperature with a specific affinity-purified anti B-50 IgGs (1/1000) obtained from serum 8920 (Oestreicher et al., 1983) (23). Finally, immunoreactivity against the primary antibody was detected by a biotinylated secondary antibody and a horseradish peroxidase-conjugated biotin-avidin complex (Vectastain Elite ABC Kit, Vector) and visualized with diaminobenzidine as chromogen.

Determination of PKC Activity. PKC activity was determined by an in vitro phosphorylation assay using histone III-S as substrate (24). The reactions were performed using 10 μg of SPM proteins in 25 μl (final volume) of 50 mM Tris-HCl buffer, pH 7.4; 0.2 mM EDTA; 10 mM $MgCl_2$; 5 mM β-mercaptoethanol; 10 μM ATP; 100 μg/ml PKA inhibitor (Sigma); 0.6 μg/μl histone H III-S and 1 μCi [γ ^{32}P] ATP, during 1 min. at 30°C. Phosphate incorporation was evaluated in the presence or absence of PKC activators: 0.1 μM phorbol ester TPA; 100 μg/ml phosphatidylserine (Sigma) and 1 mM $CaCl_2$. The reaction was terminated by addition of 12.5 μl stopping solution. The labeled phosphoproteins were separated on a 15% SDS-PAGE and following

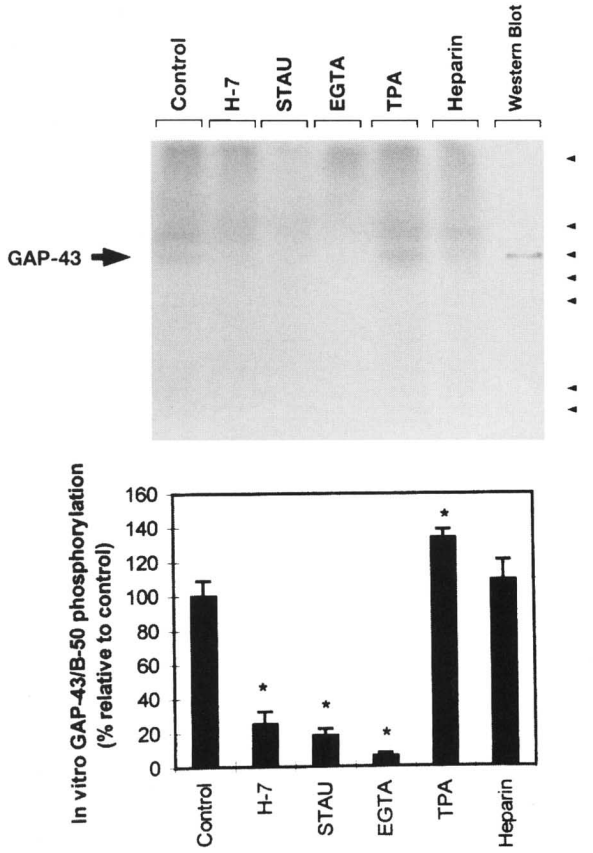

Fig. 1. Upper panel: Autoradiogram of a representative experiment showing the in vitro phosphorylation of striatal SPM proteins in the absence (control) or in the presence of different agents: H-7 (10^{-4} M) and Staurosporine (10^{-5} M), EGTA (2.5 mM), phorbol ester (TPA, 1 μM) and Heparin 5 μg/ml (Casein Kinase inhibitor). The last lane shows a representative immunoblot of GAP-43/B-50. Arrowheads indicate the position of molecular weight markers: 95.5; 55; 43; 36; 29; 18.5 and 12 KDa.

Lower panel: Quantification of the in vitro phosphorylation of GAP-43/B-50. Data are expressed as percentages of the control value and represent the mean ± SD of 3 independent determinations measured by triplicate. Significances compared with control: (*$P < 0.01$ according to Dunnett's test).

staining and destaining, the gels were dried and exposed to an AGFA-Curix film. The histone protein band was cut from the dried stained gel and the radioactivity incorporated counted in a liquid scintilliation counter. The total PKC activity was calculated as pmoles ^{32}P-incorporated per min per mg of protein above the basal activity, which was determined in the absence of PKC activators.

Statistical Analysis. A two-way analysis of variance (ANOVA) was used to analyze the results of the circling motor activity. The ANOVA design considered control vs. exercise as one factor, and ipsi vs. contralateral hemisphere as another. Differences between groups were assessed by Student's t test for unpaired and paired samples. In the experiments related to activation or inhibition of PKC, the statistical analysis was performed by Dunnett's test after one-way analysis of variance (ANOVA). The correlation coefficient (r) was obtained studying the degree of linear association between two variables using Graph Pad Instat Software Inc.

RESULTS

Endogenous phosphorylation of SPM proteins perfomed under incubation conditions which are typical for PKC, resulted in the phophorylation of numerous proteins. After one-dimensional SDS-PAGE, one of the most prominent band in the autoradiograph is that of GAP-43/B-50. Thus, in SPM the degree of GAP-43 phosphorylation can be quantified directly without immunoprecipitation (25). The identity of GAP-43/B-50 was established by electrophoretic properties on unidimensional SDS-PAGE, selective phosphorylation by PKC and specific immunoreactivity detected by western blotting (Fig. 1). Densitometric analysis of the Fig. 1 shows that in the presence of relatively specific PKC inhibitors (H7 and Staurosporine) and EGTA, the in vitro GAP-43/B-50 phosphorylation was dramatically reduced. Moreover, the phorbol ester TPA increased the endogenous phosphorylation of GAP-43/B-50 present in the same fraction (Fig. 1). PKC only phosphorylates the Ser 41 residue of GAP-43/B-50 (26,27). Furthermore, bovine GAP-43/B-50 can also be in vitro phosphorylated by Casein Kinase II (CKII), predominantly on Ser 192 and 193, but also on Thr 88, 89 and 95 (28). In order to exclude a dual participation of PKC and CKII in our preparations, we performed the phosphorylation assays in the presence of heparin, a potent inhibitor of CKII (28). Fig. 1 shows that the presence of heparin did not affect the normal level of GAP-43 phosphorylation in naive SPM. Moreover, GAP-43/B-50 phosphorylation by an unknown kinase (Ser 96 and Thr 172) has been described as sensitive to saponin detergent (29). Since the addition of this detergent to striatal SPM did not produce differences in GAP-43/B-50 phosphorylation, it suggest that this unknown kinase do not play a role here (data not shown). These results as a whole support the role of PKC in the endogenous phosphorylation of GAP-43/B-50 in SPM.

After confirmation that the studied phosphoband corresponds to GAP-43/B-50, we used similar preparations to assess whether GAP-43/B-50 endogenous phosphorylation is altered after circling motor stimulation. We isolated SPM from ipsi and contralateral striata of control and exercised animals, and subjected them to an in vitro phosphorylation assay under conditions which are typical for PKC.

As can be seen in Fig. 2, animals exercised in the giratory platform showed an average decrease of 31% in

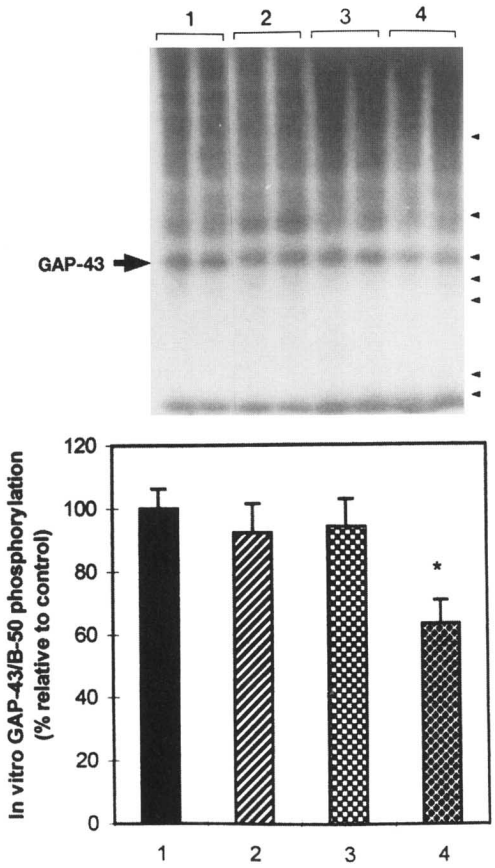

Fig. 2. Upper panel: Representative autoradiogram showing the in vitro phosphorylation of striatal SPM proteins from control and exercised rats (average speed), sacrified 30 minutes after circling motor activity. Lane 1, ipsilateral control; lane 2, ipsilateral exercised (10.2 m/min); lane 3, contralateral control and lane 4, contralateral exercised (10.2 m/min). Arrowheads indicate the position of molecular weight markers: 95.5; 55; 43; 36; 29; 18.5 and 12 KDa.
Lower panel: Quantification of GAP-43/B-50 in vitro phosphorylation. Data are expressed as percentages of the maximal control value and represent the mean ± SD of 3 independent experiments grouped according to running speeds. Each determination was assayed by quadruplicate. *P < 0.01 vs. controls by Student's *t* test for unpaired samples and vs. ipsilateral exercised by Student's *t* test for paired samples.

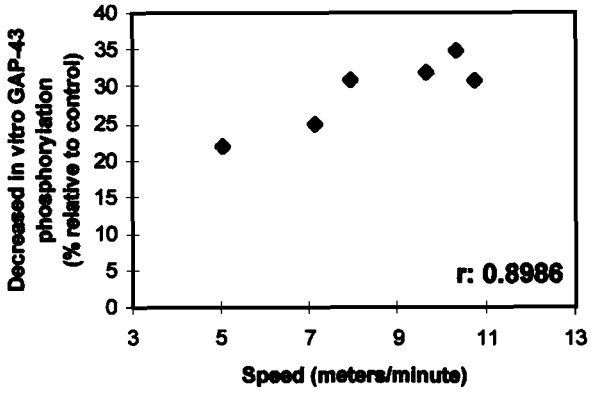

Fig. 3. Correlation between percentage of decrease of the in vitro GAP-43/B-50 phosphorylation in the striatum contralateral and motor activity (speed) acomplished during physical exercise. The animals were sacrificed 30 minutes post-exercise. P = 0.015, n = 6 independent experiments.

the level of endogenous phosphorylation of GAP-43/B-50 in the contralateral striatum with respect to the ipsilateral side also exercised (P < 0.01). Significant interhemispheric differences were not found in control animals, neither when tested left vs. right nor when striata were grouped as ipsi vs. contralateral, according to the respective exercised group. Furthermore, the circling motor stimulation produced a reduction of 33% in the level of endogenous phosphorylation of GAP-43/B-50, representing a significant difference with respect to control animals (P < 0.01). These changes were seen 30 min post-exercise in the striatum contralateral to the sense of turn, independently of the direccion of turning (clockwise or anticlockwise).

To study a possible correlation of this change with the motor performance developed by the animals during the physical activity, the rats were grouped according to their running speed (SD < 10% on each group). Plotting the percentage of drop in the in vitro phosphorylation of GAP-43 (relative to control animals) in function of motor performance (speed), we were able to find a significant correlation between these two variables (r: 0.8986, P = 0.015) (Fig. 3). In contrast to these findings, animals sacrificed immediately after motor activity (0 minutes) did not show any significant modification in the in vitro GAP-43/B-50 phosphorylation when compared with controls in both ipsilateral (+17% ± 3) and contralateral striata (−10% ± 2).

Semiquantitative immunoelectroblotting showed that the relative concentration of GAP-43/B-50 was unaltered in SPM of control and exercised animals (Fig. 4).

To test if the change in the endogenous phosphorylation of GAP-43/B-50 caused by circling motor activity could be due to a reduced PKC activity, we measured total PKC activity in the presence of an excess of histone H III-S as substrate. Fig. 5 shows the results of this set of experiments; a significant reduction of 25% (P < 0.05) in the PKC activity was observed in the contralateral striatum respect to the ipsilateral side also exercised. Significant interhemispheric differences were not found in control animals. This change was seen 30 minutes post-exercise, but not immediately after motor stimulation. There were no significant differences in kinase activity in the absence of PKC activators among

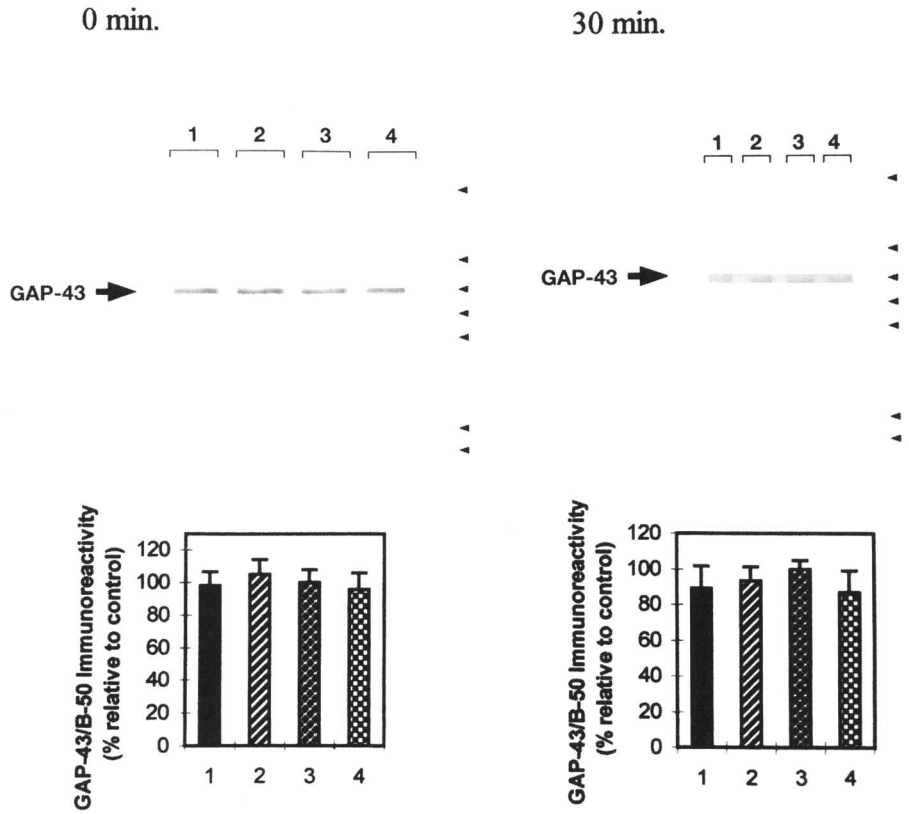

Fig. 4. Upper panel: A representative western blot analysis of GAP-43/B-50 immunoreactivity, using striatal SPM proteins (20 μg) from control and exercised animals, isolating separately the ipsi and the contralateral striatum. Lane 1, ipsilateral control; lane 2, ipsilateral exercised; lane 3, contralateral control and lane 4, contralateral exercised. In all cases, the animals were sacrificed at 0 or 30 minutes post-exercise. Arrowheads indicate the position of molecular weight markers: 95.5; 55; 43; 36; 29; 18.5 and 12 KDa.
Lower panel: Quantification of GAP-43/B-50 immunoreactivity. Data are expressed as percentages of the maximal control value and represent the mean ± SD of 4 independent determinations performed by triplicate.

the different experimental groups. These results suggest that PKC participates in the asymmetric changes detected in the exercised rat striatum.

As the reduced endogenous GAP-43/B-50 phosphorylation can be related to a decreased PKC activity, we also measured its in vitro phosphorylation in the presence of protein phosphatase inhibitors. GAP-43/B-50 dephosphorylation can be mediated by type 1 and 2A phosphatases present in presynaptic membranes (30,31) and in vitro by type 2B protein phosphatases, members of the Ca^{2+}/Calmodulin (CaM)-dependent phosphatase family, the Calcineurins (32,33). For this purpose, phosphorylation assays were performed in the presence of okadaic acid and cypermetrin to inhibit these phosphatases activities. As can be seen in Fig. 6, the circling motor stimulation maintained a reduction of 30% in the level of endogenous phosphorylation of GAP-43/B-50 in the contralateral striatum with respect to the control animals ($P < 0.05$). These results further support that the reduction of GAP-43/B-50 phosphorylation observed in contralateral striatal SPM of exercised rats is a consequence of reduced PKC activity.

DISCUSSION

Our results confirm that in striatal SPM preparations the GAP-43/B-50 is a prominent phosphorylated protein (Figs 1, 2 and 6).

Ample evidences demonstrated that the PKC is the main kinase phosphorylating GAP-43 in SPM (17,25). In recent years, several sites in GAP-43/B-50 have been shown to be phosphorylated by different kinases. PKC acts on Ser 41 (26,27), Casein Kinase II phosphorylates predominantly on Ser 192 and Ser 193, but also on Thr 88/89 and Thr 95 (bovine GAP-43/B-50) (28), whereas two other phosphorylation sites at Ser 96 and Thr 172 are substrates for an unidentified protein kinase (29).

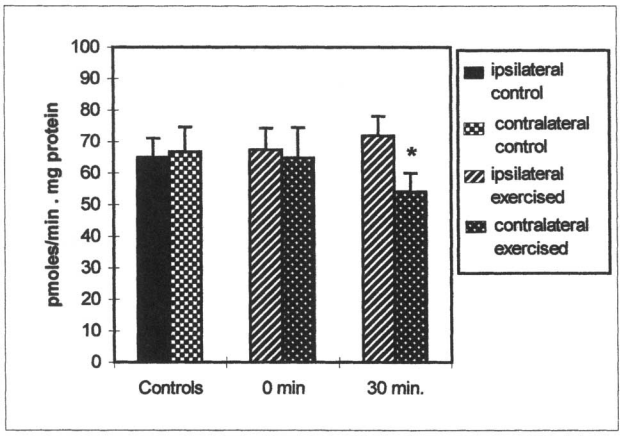

Fig. 5. Circling motor activity-related changes in PKC activity in striatal SPM from control and exercised rats. Data are expressed as pmoles of ^{32}P incorporated per min per mg protein and represent the mean ± SD of 4 independent determinations done by quadruplicate. The ^{32}P incorporation in the absence of PKC activators has been substracted. *P < 0.05 vs. ipsilateral exercised by Student's *t* test for paired samples.

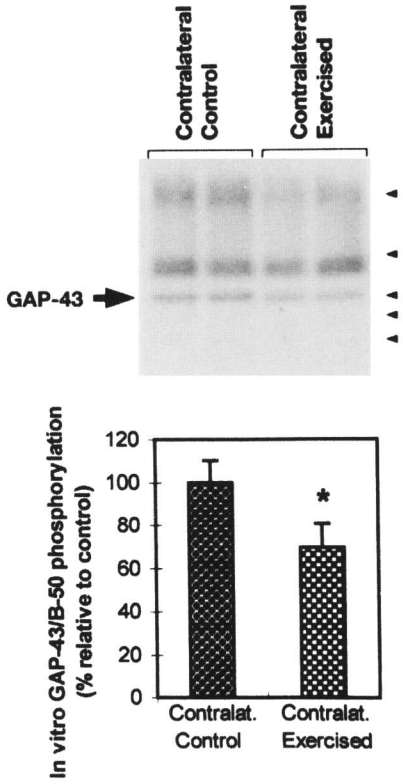

Fig. 6. Upper panel: Representative autoradiogram showing the in vitro phosphorylation of striatal SPM proteins in the presence of protein phosphatase inhibitors: cypermetrine (5 μM) and okadaic acid (2 μM). Synaptic membranes were isolated from control and exercised animals sacrificed 30 minutes after circling motor activity. Arrowheads indicate the position of molecular weight markers: 95.5; 55; 43; 36 and 29 KDa.
Lower panel: Quantification of GAP-43/B-50 in vitro phosphorylation. Data are expressed as percentajes of the control value and represent the mean ± SD of 3 independent experiments measured by duplicate. *P < 0.05 vs contralateral control by Student's *t* test for unpaired samples.

The phosphorylation by this unknown kinase has been described as sensitive to saponin detergent. Since in striatal SPM the addition of this detergent did not produce differences in GAP-43/B-50 phosphorylation, it suggest that this unknown kinase do not play a role here. In a similar way, the addition of heparin permit to exclude the participation of Casein Kinase II in our in vitro phosphorylation assay (Fig. 1). The experimental conditions used here to study in vitro phosphorylation suggest a role for PKC in our preparations of striatal membranes.

Several studies have demonstrated changes in the GAP-43/B-50 phosphorylation in different models of synaptic plasticity such as long-term potentiation, associative learning and memory formation for passive avoidance in chicks and rats (14,18,34,35). But GAP-43 can be also related to other plastic mechanisms independently of memory and learning. In the IMHV of chicken, the GAP-43 phosphorylation did not correlate with the strength of learning, but it was negatively correlated with locomotor behavior during imprinting training (18). Thus, we evaluated the role of GAP-43 in an experience-dependent motor plasticity period (9,10) subjecting the animals to motor activity in a giratory platform where learning activities are minimized in relation to motor activity, since the rats spontaneously run in the turning platform.

Our findings show a significant decrease in the endogenous phosphorylation of GAP-43/B-50 in SPM obtained from the contralateral striatum. This asymmetric change was found at 30 minutes post-running (Fig 2). The correlation between in vitro GAP-43 phosphorylation decrease (relative to control) and the running speed acomplished during the physical activity, supports that the observed changes in phosphorylation are related to the motor activity of the animals. Phosphorylation assays performed in the presence of heparin, did not altered the differences observed in GAP-43/B-50 phosphorylation between control and exercised striata (data not shown). Since the level of GAP-43/B-50 in SPM was similar in all preparations, the decrease in the phosphorylation might be the consequence of a reduced amount of membrane-bound protein kinase, a reduced concentration of endogenous activators (i.e. diacylglycerols, phosphatidylserine or arachidonic acid) or an increased activity of phosphoprotein phosphatases.

Specifically, it has been shown that activation of D1 and D2 Dopamine receptors exert a direct, inhibitory effect on particulate PKC activity in rat striatum (36,37). However, in the presence of calcium, the D1 agonists yielded the opposite alteration in PKC activities in this same preparation. These results support a role for Dopamine receptor-mediated modulation of PKC phosphorylation (37).

To determine if the drop in the endogenous phosphorylation of GAP-43/B-50 could be due to a reduced PKC activity, we measured the activity of this kinase in control and exercised animals. These results showed the PKC participation in the asymmetric changes detected in the exercised rat striatum and suggest that the decreased GAP-43/B-50 phosphorylation is related to a reduced contralateral PKC activity in SPM. Moreover, phosphorylation assays carried out in the presence of phosphatase inhibitors support a reduced contralateral kinase activity.

It has been postulated that GAP-43 phosphorylation increases neurotransmitter release (15,17). In contrast to this, we have found a reduction of in vitro GAP-43/B-50 phosphorylation, that, if it is paralleled in vivo, it would indicate a decrease of striatal neurotransmission. In Post-hoc phosphorylation assays it is generally assumed that a decrease in the in vitro uptake of ^{32}P might be interpreted as increased phosphorylation in vivo. However, it may also reflect a reduced phosphorylation of the substrate in vivo. Interestingly, in studies on synaptic protein phosphorylation and LTP, an increased in vivo labeling of GAP-43/B-50 phosphorylation was observed by Gianotti and colleagues (38). These in vivo data parallel the findings of increased in vitro GAP-43/B-50 after LTP, using an identical assay as the one used in the present study (39). This suggests that the reduced endogenous phosphorylation of GAP-43/B-50 observed in vitro in our work (Fig 2) probably reflects a decrease in the in vivo phosphorylation level. A point of concern is that GAP-43 modification was found 30 minutes post-exercise, but not immediately after motor activity was performed. Thus, it is unlikely that GAP-43 alteration is related to motor activity per se but it could play a modulatory role in the plastic events triggered after completion of motor stimulation. In addition, a role for GAP-43 phosphorylation in priming or modulation of neurotransmitter release in rat striatum after AMPH treatment has been proposed by Gnegy et al (17).

Recently, we have demonstrated that the circling motor behavior, in an operant conditioning test, triggers a long term reduction in the levels of striatal muscarinic and dopaminergic D2 receptors, only when the animals were trained at 30 days of age (9,10).

Due to the role of GAP-43 in synaptic plasticity, the above reported modifications in GAP-43 phosphorylation could be related to the long term alterations of neuroreceptors in rat striatum during the postnatal critical period. However further experiments are required to determine whether the variation in GAP-43/B-50 is involved in the induction of the permanent changes of striatal neuroreceptors. It is possible that some of the effects produced by circling motor activity are mediated via an altered phosphoinositide turnover because activation of Dopamine D2 receptors in vitro was shown to decrease the levels of $[^{32}P]PIP_2$ in striatal slices and synaptosomal membranes (40,41), but had not effect on phosphoinositide hydrolysis (42). However, the molecular mechanism by which the D1 and D2 drugs alter PKC activity reflects a change in the Km value for calcium similar to those induced by diacylglycerol, which is produced from the hydrolisis of the polyphosphoinositides during signal transduction. It is therefore possible that the D1 and D2-acting drugs alter the kinetic properties of particulate PKC via a change in PI turnover and diacylglycerol generation within the cell. Itself, the degree of GAP-43/B-50 phosphorylation has been reported to modulate the activity of the PIP-kinase, the lipid kinase that phsophorylates PIP to PIP_2 (43,44). The availability of PIP_2 itself may be a PKC activator (45) and thus the regulation of the amount of PIP_2 through the phosphorylation states of GAP-43/B-50 could be of importance for the regulation of transmitter release. Thus, a decrease in GAP-43/B-50 phosphorylation could be related with a reduced activity of striatal dopaminergic and cholinergic pathways, two neurotransmitter systems that are highly interconnected and which adequate interaction is crucial to facilitate the modulatory role of the striatum in motor performance. At this respect, stimulation of GAP-43/B-50 phosphorylation has been correlated with mAChR activation (46).

The reported changes occurred at the time of striatal development in which gross functional and morphological maturation of the striatum has been accomplished (47), and synaptic functional adjustment could be taking place in the striatal modulatory neurotransmission systems as a consequence of physiological motor activity. The possibility of connections with differential activity-dependent plasticity at the end of morphogenesis has been proposed recently by Singer (48) for sensory cortices. It is open to further research if a comparable description is possible in a subcortical motor structure, like the striatum.

Finally, a conflicting point arose in relation to the above suggested participation of GAP-43 in the long term plastic changes in striatal cholinergic receptors.

GAP-43 modification is asymmetrical, while delayed cholinergic receptor reduction is clearly not. At least two hypotheses can be mentioned: (a) decreased GAP-43 phosphorylation in presynaptic terminals is not strictly related to postsynaptic receptor changes, or (b) the temporal sequence of molecular events that leads to permanent plastic modifications goes beyond the 30 minutes tested in the present work and compensatory mechanisms appear in the ipsilateral striatum.

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