Evidence for Protein Kinase C Involvement in the Short-Term Activation by Prolactin of Tyrosine Hydroxylase in Tuberoinfundibular Dopaminergic Neurons

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Abstract: The mechanism of the short-term activation by prolactin (PRL) of tyrosine hydroxylase (TH) in tuberoinfundibular dopaminergic neurons was examined in vitro on hypothalamic slices from ovariectomized rats. TH activity (determined by 3,4-dihydroxyphenylalanine accumulation within 2 h of incubation of the hypothalamic slices with PRL) showed a dose-dependent increase in the median eminence after blockade of decarboxylase inhibitors. TH activity increased to 29.92 ± 0.49 µM, the other being 1.96 ± 0.09 µM, likely corresponding to a phosphorylated and active form and to a nonphosphorylated and less active form, respectively. After PRL treatment, the TH form of low K(DA), remained unaffected, whereas the TH(DA) of the purported active form of TH increased to 62.6 ± 0.8 µM, suggesting an increase in the enzyme phosphorylation. This increase in the K(DA) TH was selectively prevented by GF 109203X, a potent and selective inhibitor of protein kinase C, but not by a specific inhibitor of protein kinase A or calmodulin. Finally, this action of PRL could be mimicked by 12-0-tetradecanoylphorbol 13-acetate (a direct activator of protein kinase C). These results suggest that PRL, at the median eminence level, activates TH by increasing the enzyme phosphorylation and that this action may involve an activation of protein kinase C. Key Words: Tyrosine hydroxylase—Dopamine feedback inhibition—Phosphorylation—Prolactin—Tuberoinfundibular neurons—Protein kinase C.


Pituitary prolactin (PRL) is known to feed back to the tuberoinfundibular dopaminergic (TIDA) neurons to regulate the release of dopamine (DA; Gudelsky and Porter, 1980) and, ultimately, its own release. Evidence for an autoregulatory action of PRL on its secretion was first reported by Clemens and Meites (1968). It was later demonstrated that the DA turnover in the median eminence (ME) of rats was elevated by PRL treatment (Hökfelt and Fuxe, 1972), suggesting that the PRL feedback is mediated, at least in part, by tuberoinfundibular DA.

Stimulation of TIDA neuronal activity has been supported by many subsequent studies: PRL is known to stimulate DA synthesis (Perkins et al., 1979; Chen and Ramirez, 1989) and turnover (Hökfelt and Fuxe, 1972; Selmanoff, 1981) in tuberoinfundibular neurons as well as DA release into hypophysial portal blood (Gudelsky and Porter, 1980) and from isolated nerve endings (Perkins and Westfall, 1978; Foreman and Porter, 1981; Felman and Tappaz, 1990). However, the mechanism(s) by which PRL effects these changes remains unknown.

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the catecholaminergic biosynthetic pathway (Levitt et al., 1965) whose activity can be regulated by both modulation of its synthesis and by activation/deactivation of the existing enzyme molecules (for review, Zigmond, 1988-89; Zigmond et al., 1989; Filenz, 1990). There is evidence that in the TIDA neurons, PRL can stimulate TH activity (Chen and Ramirez, 1989). However, Demarest et al. (1984) clearly showed that there are two components in the PRL-induced activation of TIDA neurons; i.e., a sluggish one is observed after a latent period of 12–24 h after elevation of PRL and corresponds to an "induction" component in response to a prolonged elevation.
in PRL concentrations. This delayed PRL-induced increase in TH activity requires ongoing protein synthesis, presumably new molecules of TH, because PRL has been shown later on to increase TH gene expression selectively in the arcuate nuclei (Arbogast and Voogt, 1991). The other component is observed within 4 h of elevation in PRL concentrations and is not prevented by cycloheximide, an inhibitor of protein synthesis (Demarest et al., 1984). The mechanism underlying this rapid PRL-induced activation of TH is the subject of the present investigation.

We have previously shown in vivo (Pasqualini et al., 1991) that 2 h after an estradiol-induced PRL surge, TH activity increased markedly and that this increase could be prevented by the pretreatment of rats with a specific rat PRL antiserum. It is interesting that at the time of maximal TH activity, the number of TH molecules in the ME was reduced (Pasqualini et al., 1991). For this reason, a rapid effect of PRL on the catalytic properties of the remaining molecules of TH was suggested. Because short-term regulation of TH is mainly effected by phosphorylation/dephosphorylation reactions, it was likely that the mechanism by which PRL activates TH involved such a covalent modification of the enzyme. However, four phosphorylation sites appear to be involved in the phosphorylation of TH in intact rat tissues (Ser4, Ser19, Ser31, and Ser40), and physiological stimuli increase 32P incorporation into the latter three (Haycock, 1990; Haycock and Haycock, 1991). The phosphorylation of these three sites is regulated by the following three distinct intracellular signaling pathways in rat catecholaminergic tissues (Haycock, 1993): Ser19 via calcium/calmodulin-dependent protein kinase (PKC)–extracellular signal regulated kinases (ERKs), and Ser40 via cyclic AMP (cAMP)-dependent protein kinase (PKA).

Thus, the objectives of this study were to determine (1) whether an increase in TH level of phosphorylation was involved in the short-term stimulation of TH activity by PRL, and (2) which protein kinase could eventually mediate this PRL effect.

To this end, we studied an index of TH catalytic activity that is very sensitive to the state of TH phosphorylation (Ames et al., 1978; Lazar et al., 1982; Albert et al., 1984; Fujisawa and Okuno, 1986), i.e., the enzyme’s constant of inhibition by DA \( K_{i,DA} \), which characterizes its sensitivity to end-product feedback inhibition.

**MATERIALS AND METHODS**

**Animals**

Female Wistar rats (200–220 g, Iffa Credo, Lyon, France) were housed under controlled temperature (22°C) and lighting (on from 0500 to 1900 h) and supplied with water and food ad libitum. All rats were bilaterally ovariec-
tomized and used 11–13 days after surgery.

**Chemicals**

H-89 and GF 109203X were obtained from Calbiochem, dissolved in dimethyl sulfoxide (DMSO) as stock solutions and stored at −30°C. N-(6-Aminohexyl)-5-chloro-1-naphthalenesulfonamide (W7) and N',2'-O-dibutyryl cAMP (dibutyryl cAMP, the liposoluble cAMP derivative) were obtained from Sigma and dissolved extemporaneously in the incubation medium. Staurosporine as well as the phor-

**Hypothalamic slices preparation and treatment**

Animals were decapitated between 1000 and 1130 h. From each animal, a sagittal hypothalamic slice including the ME, arcuate nucleus, and small parts of the ventromedial and periventricular nuclei was prepared as described by Arita and Kimura (1984). The tissue was equilibrated under an atmosphere of 95% O2/5% CO2 for 15 min at 37°C in 1 ml of Earle’s balanced salt solution (EBSS; pH 7.4) containing 50 μM tyrosine. The preincubation medium was then removed, and 500 μl of EBSS containing or lacking prolactin was added. Prolactin of ovine (NIDDK oPRL-19) or of rat (NIDDK rPRL-1-6) origin was first solubilized in 0.01 M sodium bicarbonate.

**In vitro 3,4-dihydroxyphenylalanine (DOPA) accumulation**

In the first series of experiments, in vitro DA biosynthesis in TIDA neurons was estimated by a previously described method (Arita and Kimura, 1984) measuring the DOPA accumulated in the ME after incubation of hypothalamic slices with NDS 1055 (broresine; 4-bromo-3-hydroxybenzylxoxamine; generous gift of Dr. J. G. B. Howes, Smith and Nephew Research Limited, Harlow, Essex, U.K.). This specific aromatic L-amino acid decarboxylase inhibitor (Chalfie and Perlman, 1977) was added in the medium 40 min before the end of incubation, at a final concentration of 200 μM. The MEs were then dissected out with fine scissors under a microscope, homogenized in 60 μl of 0.1 M perchloric acid containing 0.005% EDTA-Na2, and finally centrifuged at 15,000 g for 10 min. The pellets were solubilized in 0.1 M sodium hydroxide and analyzed for protein content by the bicinchoninic acid protein microas-

**Inhibition studies with D4.** For kinetic studies, TH activity was determined by measuring the amount of exogenous tyrosine converted to DOPA in vitro by ME homogenates, as follows: In this series of experiments, the MEs were first dissected out; they were equilibrated under an atmosphere of 95% O2/5% CO2 for 15 min at 37°C in 1 ml of EBSS. After removal of this preincubation medium, they were incubated in 500 μl of EBSS containing or not containing PRL (alone or in the presence of H-89, W7 or GF 109203X or the vehicle (0.25% DMSO), or TPA), and, for the last series of experiments, in 500 μl of EBSS containing or not containing dibutyryl cAMP. H-89, with selectivity and selective inhibitor of PKA \( K_{i} = 48 ± 8 \) nM; Chiu and Garthwaite (1990) was used at 100 nM, a concentration that effectively blocks a dibutyryl cAMP-induced activation of TH in the ME (C. Pasqualini et al., unpublished results). W7 was used at 100 μM, a concentration that has been shown to inhibit a Ca2+-calmodulin-dependent activation of TH in tissue slices.
were solubilized in 0.1 M sodium hydroxide and analyzed for protein content as indicated above. TH activity in the supernatants was immediately assayed as described previously (Leviel et al., 1990) with minor modifications, as follows. Incubation was performed at 37°C for 15 min, with 0.1 or 0.5 mM \( \alpha \)-methyl DOPA (10 ng) was added in each sample as an internal standard, the homogenates were centrifuged at 15,000 g for 15 min. The DOPA content of the supernatants was quantitated in each experiment.

Duplicates were run on all experimental points. The TH activity of hypothalamic slices with PRL treatment of hypothalamic slices with PRL was determined at 10-20 rats. Statistical significance was assessed by Student’s \( t \) test.

RESULTS

TH catalytic activity in the ME after in vitro treatment of hypothalamic slices with PRL

TH activity was determined by the rate of in vitro accumulation of DOPA in the ME after inhibiting the activity of aromatic L-amino acid decarboxylase with the addition of increasing concentrations of DA (1-50 \( \mu \)M). To stop the reaction, 50 \( \mu \)l of 1 M HC104 containing 5 mM sodium metabisulfite and 1.5 mM EDTA-Na, was added. After \( \alpha \)-methyl DOPA (10 ng) was added in each sample as an internal standard, the homogenates were centrifuged at 15,000 g for 15 min. The DOPA content of the supernatants was quantitated as indicated above.

Because PRL is known to stimulate DA release from the ME, the concentration of endogenous DA contained in the supernatants from controls and PRL-treated MEs was quantitated in each experiment (at 0 \( \mu \)M of added DA). No significant difference could ever be found between the two groups, i.e., values were 0.395 ± 0.06 and 0.402 ± 0.05 \( \mu \)M, respectively, for controls and PRL-treated supernatants (both mean ± SEM of 10 independent determinations).

In the control MEs, the values for TH activity obtained at 0.1 and 0.5 mM 6-MPH4 could be resolved into two straight lines, as follows: (1) On the basis of the data obtained with the higher DA concentrations (Fig. 2, top, extrapolated broken lines), the \( K_i \) value for DA was calculated to be 29 \( \mu \)M. (2) On the basis of the data obtained with the lower concentrations of DA, a second \( K_i \) value was calculated to be 2 \( \mu \)M. Thus, in the control MEs, TH appeared to exist as two kinetically different forms, with \( K_i \) values of 29.92 ± 0.49 and 1.96 ± 0.09 \( \mu \)M (both mean ± SEM of 12 independent determinations).

In the MEs treated with PRL (10 \( \mu \)g/ml) for 2 h, two kinetically different forms of TH also coexisted; whereas the TH form exhibiting a \( K_i \) value of 2 \( \mu \)M was still observed, the other one underwent an increase by approximately twofold of its \( K_i \) value (from 29 to 62 \( \mu \)M). The same result was obtained when rat PRL was used. On the contrary, when the MEs were incubated for only 30 min with PRL, no change in the \( K_i \) value of TH could be observed (data not shown).

*Fig. 1. Effect of oPRL on in vitro DOPA accumulation in the ME. Hypothalamic slices were incubated for 2 h in EBSS lacking (C) or containing increasing concentrations of oPRL or 10 \( \mu \)g/ml of BSA. NSD 1055 was added to the medium 40 min before the end of incubation. Each value is a mean ± SEM of determinations from 10-20 rats. *\( p < 0.01; ***p < 0.001, \) vs. the control.*
Effect of protein kinase inhibitors on the PRL-induced change in TH susceptibility to DA inhibition and in TH activity

In this series of experiments, TH activity was determined by measuring the quantity of DOPA formed at 0 \( \mu M \) of added DA, in the presence of 0.5 mM 6-MPH4, and was expressed as a percentage of control values (see Table 1).

In the presence of H-89, a specific PKA inhibitor, the effect of PRL on TH \( K_i(DA) \) was not prevented (Fig. 3). When the MEs were first incubated with H-89 (100 nM) alone for 5 min and then in the absence (Fig. 3, top) or presence (Fig. 3, bottom) of oPRL (10 \( \mu g/ml \)) for another 2 h, the patterns of the Dixon plots of TH activity were similar to the ones observed in the absence of H-89. Likewise, H-89 had no effect on basal or PRL-stimulated TH activity (Table 1).

Similarly Fig. 4 shows that, even in the presence of W7, a calmodulin inhibitor (Hidaka et al., 1981), PRL still exerted its effect on TH susceptibility to DA. When the MEs were incubated with W7 (100 \( \mu M \)) alone for 5 min and then in the absence (Fig. 4, top) or presence (Fig. 4, bottom) of oPRL (10 \( \mu g/ml \)) for another 2 h, the patterns of the Dixon plots of TH activity were similar to the ones observed in the absence of W7 (Fig. 2).

By contrast, PRL effects on TH activity and \( K_i \) for DA were selectively prevented by the following two potent inhibitors of PKC: (1) staurosporine, which, at a low concentration, preferentially inhibits PKC (Tamaoki et al., 1986), and (2) GF 109203X, which is much more selective than staurosporine (Toullec et al., 1991). When the MEs were first incubated with staurosporine (5 nM, data not shown) or GF 109203X (5 nM) alone for 5 min and then in the absence (Fig. 5, top) or presence (Fig. 5, bottom) of oPRL for another 2 h, the effect of PRL on the \( K_i(DA) \) value of TH was abolished. Likewise, though without

**TABLE 1. Effect of protein kinase inhibitors on basal and PRL-stimulated TH activity in the ME**

<table>
<thead>
<tr>
<th>Addition to the incubation</th>
<th>TH activity (nmol DOPA/h/mg prot.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100 ± 5.9</td>
</tr>
<tr>
<td>PRL</td>
<td>180.3 ± 10.4*</td>
</tr>
<tr>
<td>H-89</td>
<td>101 ± 11.9</td>
</tr>
<tr>
<td>H-89 + PRL</td>
<td>178 ± 7.1*</td>
</tr>
<tr>
<td>W7</td>
<td>103 ± 12</td>
</tr>
<tr>
<td>GF 109203X</td>
<td>109 ± 14</td>
</tr>
<tr>
<td>GF 109203X + PRL</td>
<td>96 ± 12.5</td>
</tr>
</tbody>
</table>

Values are expressed as a percentage of the control values, based on 10-12 determinations.

* \( p < 0.001 \), vs. the control value.

FIG. 3. Lack of effect of the PKA inhibitor H-89 on the PRL-induced change in TH susceptibility to DA inhibition. Dixon plots of TH activity as a function of DA and 6-MPH concentrations, in MEs incubated for 5 min with H-89 (100 nM) alone and then for another 2 h in the absence (top) or presence (bottom) of oPRL, as compared with the PRL-induced change without H-89 (middle). Data are values for duplicate samples from a typical experiment replicated twice with similar results.

Effect alone, GF 109203X blocked the PRL-induced increase in TH activity (Table 1).

Effect of protein kinase activators on TH susceptibility to DA inhibition

To confirm that the action of PRL on the $K_{i(DA)}$ value of TH could be attributed to the activation of PKC, the effects of a phorbol ester (known to be a potent and direct activator of this kinase; Castagna et al., 1982) and PRL, alone or in combination, were compared. As shown in Fig. 6, the treatment of the MEs with TPA (100 nM) for 2 h (middle) promoted the same change in the $K_{i(DA)}$ value of TH as PRL (see Fig. 2), an effect that was blocked by GF 109203X, a specific inhibitor of PKC (data not shown). No further change was observed when TPA and PRL were present at the same time in the incubation medium of the MEs (Fig. 6, bottom), suggesting that the same mechanism could be involved in TPA and PRL action.

PRL (or TPA) stimulation never affected the 2 $\mu M$ $K_{i(DA)}$ form of TH. Because PRL and TPA elicited only moderate (80 ± 10 and 72 ± 13%, respectively) increases in TH activity, a treatment known to maximally activate TH was then tested. The MEs were incubated in the presence of dibutyryl cAMP (2 mM, for 2 h) to stimulate PKA activity, a treatment that, in our hands, produced a 400–500% increase in TH activity (data not shown). As expected, this treatment increased (by approximately fourfold, from 31 to 117 $\mu M$) the $K_{i(DA)}$ value of the purported active form of TH but also left the 2 $\mu M$ $K_{i(DA)}$ form unaffected (Fig.

FIG. 4. Lack of effect of the calmodulin inhibitor W7 on the PRL-induced change in TH susceptibility to DA inhibition. Dixon plots of TH activity as a function of DA and 6-MPH₄ concentrations, in MEs incubated for 5 min with W7 (100 μM) alone and then for another 2 h in the absence (top) or presence (bottom) of oPRL (10 μg/ml). Data are values for duplicate samples from a typical experiment replicated twice with similar results.

7, bottom). Thus, it was interesting to determine whether this form was really not phosphorylatable or rather simply not accessible to the kinases because of its cellular distribution. To this aim, a cAMP-dependent protein phosphorylation was performed after protein solubilization by Triton X-100 (exactly as described by Iuvone and Dunn, 1986) of the ME homogenates and, in this case, an increase in the \( K_i \) values of all TH molecules was indeed observed (data not presented).

**DISCUSSION**

The present study demonstrates that in vitro treatment of hypothalamic slices containing TIDA neurons with physiological doses of PRL increases TH activity in the ME. These data confirm and extend previous in vivo results showing an increase in DA synthesis in the mediobasal hypothalamus of male rats within 4 h of a serum PRL elevation induced either by the administration of the neuroleptic trifluoperazine (Perkins et al., 1979) or by the subcutaneous injection of oPRL (Chen and Ramirez, 1989). We could actually observe this increase within 2 h of in vitro PRL treatment. This latency is in agreement with that we observed between the maximal serum release of PRL, induced in ovariectomized rats by estradiol, and the increase in TH activity in the ME (Pasqualini et al., 1991). The concentrations of PRL used in the present experiments were in the range of those actually found in the portal blood delivered to the ME in the adult male rat, i.e., >5 μg/ml (Oliver et al., 1977). Besides, these data show that PRL acts directly on the mediobasal hypothalamus to trigger this activation of TH.

The present data also indicate for the first time that the PRL-induced activation of TH in the ME involves a change in this enzyme phosphorylation, as evidenced by an increase in the \( K_i \) value of TH for DA after PRL treatment of the MEs. The catecholamines, end product of the biosynthetic pathway, function as feedback inhibitors by competing with the pterin cofactor (Udenfriend et al., 1965; Ikeda et al., 1966; Udenfriend, 1966; Nagatsu, 1973). One of the primary effects of phosphorylation in vivo is to override end-product feedback inhibition by an increase in the \( K_i \) value of TH for DA (Ames et al., 1978; Mann and Gordon, 1979; Fujisawa and Okuno, 1986). Thus, short-term regulation of TH appears to be primarily mediated by a change in affinity for inhibitory catecholamines (Ames et al., 1978; Okuno and Fujisawa, 1985; Haavik et al., 1990). In fact, phosphorylation increases the off rate of the Fe(III)-bound catecholamines from TH (Haavik et al., 1990) and thus allows all the iron to participate in catalysis (Andersson et al., 1992).
In the present study, as well as in a previous one (Pasqualini et al., 1993), it appeared that in the ME of control ovariectomized rats, TH exists in two kinetically different forms, one being ≈15-fold more sensitive to DA inhibition (K_{i(DA)} = 1.96 ± 0.09 μM) than the other (K_{i(DA)} = 29.92 ± 0.49 μM). In light of the K_{i(DA)} values reported by Vrana et al. (1981) for nonphosphorylated (K_i = 4.5 μM) and phosphorylated (K_i = 28 μM) striatal TH, we hypothesized that in the untreated MEs, the TH form exhibiting a K_{i(DA)} value of ≈30 μM could be a phosphorylated and active form, whereas the one exhibiting a K_{i(DA)} of ≈2 μM could be a nonphosphorylated and less active form. Accordingly, we have recently shown (Pasqualini et al., 1993) that estradiol, which reduces ME TH activity, does convert the TH form displaying a ≈30 μM K_{i(DA)} value into one with an ≈2 μM K_{i(DA)} value, an action that is selectively prevented by okadaic acid, an inhibitor of protein phosphatases 1 and 2A, which are the major TH phosphatases in vivo (Haavik et al., 1989).

PRL treatment was presently shown to decrease by approximately twofold the sensitivity of the purported active form of TH to DA inhibition, by converting the TH molecules with a high K_{i(DA)} value into ones with a twofold higher K_{i(DA)} value. This change in the kinetic state of TH is quite similar to that observed by others after cAMP- or Ca^{2+}/phospholipid-dependent phosphorylation of TH purified from rat striatum (Ames et al., 1978; Vrana et al., 1981; Lazar et al., 1982; Albert et al., 1984). Thus, PRL-stimulated TH is relieved from end-product feedback inhibition, an effect that is characteristically brought up by phos-
phorylation. The idea that PRL has triggered an increase in TH phosphorylation is further strengthened by the fact that when (PKC-dependent) phosphorylation was blocked, PRL could no longer alter the $K_{i(DA)}$ value of TH.

To determine why the $2 \mu M K_{i(DA)}$ form of TH was not affected by PRL stimulation, a treatment known to elicit a maximal activation of the enzyme was also tested, i.e., stimulation of PKA by dibutyryl cAMP to increase phosphorylation of Ser40 (Haycock, 1993). This treatment (which produced a four- to fivefold increase in TH activity) also left the $2 \mu M K_{i(DA)}$ TH form unaffected, although increasing by fourfold the $K_{i(DA)}$ value of the other one.

The question thus arose whether this form was a species of TH not phosphorylatable because of a small difference in structure, or rather a form simply not accessible to the kinases because of its cellular distribution. In fact, the coexistence of two kinetically different forms of TH had already been reported; Kuczenski and Mandell (1972) showed that in rat striatum TH exists as a soluble and a particulate form, exhibiting a significantly different affinity for pterin cofactor (DMPH$_4$) and sensitivity to DA inhibition. Thus, the difference in kinetic properties may be related to a difference in the physical form. In line with this idea, Coyle (1972) showed that in the absence of a detergent in the brain homogenization buffer, 40% of TH activity is not released. Finally, Morita et al. (1987) showed that a fraction of adrenal TH is associated with the membranes of chromaffin granules and that this enzyme is less active than the soluble

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**FIG. 6.** Activation of PKC by TPA mimics the PRL-induced change in ME TH susceptibility to DA inhibition. Dixon plots of TH activity as a function of DA and 6-MPH$_4$ concentration in MEs incubated for 2 h in the absence (top) or presence of TPA (100 nM) alone (middle) or together with oPRL (10 ng/ml; bottom). Data are values for duplicate samples from a typical experiment replicated twice with similar results.
form. Thus, we hypothesized that the 2 μM \( K_{\text{DA}} \) form could correspond to TH molecules associated to macromolecular cell components and thus not readily accessible to regulation. This hypothesis was strengthened by the fact that, when performed after the solubilization of TH, the cAMP stimulation triggered an increase in the \( K_{\text{DA}} \) values of all TH molecules. This latter result does not prove, but is consistent with the hypothesis, that in situ only soluble TH may be submitted to this mechanism of regulation.

Our present data also show that PRL acts directly on the ME to trigger this increase in TH phosphorylation, because it was observed even when isolated MEs were incubated in the presence of the hormone. The origin of the high concentration of PRL in the portal blood delivered to the ME is likely to be multiple; PRL is produced by the anterior pituitary and may reach the ME by way of retrograde blood flow in the hypophysial portal vessels (Oliver et al., 1977), but PRL may also be produced by hypothalamic (Schachter et al., 1984) or even extra hypothalamic (Valatx et al., 1992) neurons. Whatever the relative importance of the sources, the presently described effect of PRL is likely to be mediated by the specific binding sites for this hormone, which have been evidenced in the ME (Barton et al., 1989; Crumeyrolle-Arias et al., 1993).

If some PRL targets are now identified, the mechanism of its numerous physiological actions remain obscure. Although there is no consensus regarding the second messenger(s) that may mediate the actions of PRL, PKC has been shown to be a potential candidate and its activation has been associated with some PRL actions in the Nb2 T-lymphoma cells, mammary gland, and liver (see Meyer et al., 1992). Moreover in the hypothalamus, PRL has been shown to activate PKC; i.e., in vivo and in vitro, physiological concentrations of PRL cause a rapid translocation of a PKC from the cytosol to the membrane (DeVito et al., 1991). Accordingly, we showed that PRL action on the \( K_{\text{DA}} \) value of TH was selectively abolished by a specific inhibitor of PKC and also was mimicked by a direct activator of the same kinase. These findings are thus consistent with the view that, in the ME also, PRL may activate PKC.

Agents that activate PKC, such as phorbol esters, are known to selectively increase TH phosphorylation at Ser\(^3\) (Haycock, 1990; Haycock et al., 1992). Actually, PKC is upstream in a pathway leading to the phosphorylation of Ser\(^3\) by ERKs, a family of microtubule-associated protein and myelin basic protein kinases. Thus, activation of PKC precedes the downstream activation of ERK and Ser\(^3\) phosphorylation, which is characteristically sluggish and produces minimal effect upon TH activity (Haycock et al., 1992). In agreement, the presently described effect of PRL on the \( K_{\text{DA}} \) value of TH was rather sluggish in onset (>30 min) and produced a moderate (80%) increase in TH activity. These results are thus consistent with the hypothesis that increased TH activity in situ that is associated with a PRL elevation might result from a PKC/ERK-dependent TH phosphorylation at Ser\(^3\).

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**FIG. 7.** Effect of PKA stimulation on TH susceptibility to DA inhibition. Dixon plots of TH activity as a function of DA and 6-MPH\(_4\) concentrations, in MEs incubated for 2 h in the absence (top) or presence (bottom) of dibutyryl cAMP (2 mM). Data are values for duplicate samples from a typical experiment replicated twice with similar results.
In conclusion, we have provided evidence that PRL activation of ME TH involves a decrease in TH susceptibility to DA inhibition, presumably due to the phosphorylation of the enzyme, an effect that could be mediated by an activation of PKC.

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REFERENCES


