Stoichiometry of Tyrosine Hydroxylase Phosphorylation in the Nigrostriatal and Mesolimbic Systems In Vivo: Effects of Acute Haloperidol and Related Compounds

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Abstract: Electrical stimulation of the medial forebrain bundle increases $^{32}$P incorporation into striatal tyrosine hydroxylase (TH) at Ser$^{19}$, Ser$^{31}$, and Ser$^{40}$. In the present studies, the effects of acute haloperidol and related drugs on site-specific TH phosphorylation stoichiometry (PS) in the nigrostriatal and mesolimbic systems were determined by quantitative blot immunolabeling using phosphorylation state-specific antibodies. The striatum (Str), substantia nigra (SN), nucleus accumbens (NAc), and ventral tegmental area (VTA) from Sprague–Dawley rats were harvested 30–40 min after a single injection of either vehicle, haloperidol (2 mg/kg), raclopride (2 mg/kg), clozapine (30 mg/kg), or SCH23390 (0.5 mg/kg). In vehicle-injected control rats, Ser$^{19}$ PS was 1.5- to 2.5-fold lower in Str and NAc than in SN and VTA, Ser$^{31}$ PS was two- to fourfold higher in Str and NAc than in SN and VTA, and Ser$^{40}$ PS was similar between the terminal field and cell body regions. After haloperidol, Ser$^{40}$ PS increased twofold in Str and NAc, whereas a smaller increase in SN and VTA was observed. The effects of haloperidol on Ser$^{19}$ PS were similar to those on Ser$^{40}$ in each region; however, haloperidol treatment increased Ser$^{31}$ PS at least 1.6-fold in all regions. The effects of raclopride on TH PS were comparable to those of haloperidol, whereas clozapine treatment increased TH PS at all sites in all regions. By contrast, the effects of SCH23390 on TH PS were relatively small and restricted to the NAc. The stoichiometries of site-specific TH phosphorylation in vivo are presented for the first time. The nigrostriatal and mesolimbic systems have common features of TH PS, distinguished by differences in TH PS between the terminal field and cell body regions and by dissimilar increases in TH PS in the terminal field and cell body regions after acute haloperidol. Key Words: Clozapine—Raclopride—SCH23390—Catecholamine—Labile epitopes.


The phosphorylation of tyrosine hydroxylase (TH) is an established physiological mechanism of TH activation. Increases in phosphorylation at Ser$^{19}$, Ser$^{31}$, and Ser$^{40}$ occur after depolarizing stimuli in vivo (Haycock and Haycock, 1991) or in situ (Waymire et al., 1988; Haycock, 1990; Mitchell et al., 1990; Thomas et al., 1997). However, the phosphorylation of TH at each site is associated with distinct signaling pathways. Conditions that increase Ca$^{2+}$/calmodulin-dependent protein kinase II have been shown to phosphorylate TH in vitro (Vulliet et al., 1984); however, its involvement in Ser$^{19}$ phosphorylation in situ has not been established. Treatments that affect extracellular signal-regulated protein kinase signal transduction increase Ser$^{31}$ phosphorylation in situ (Haycock, 1990, 1996). Extracellular signal-regulated protein kinase phosphates Ser$^{31}$ in vitro and in situ (Haycock et al., 1992; Sutherland et al., 1993). Agents that increase intracellular cyclic AMP (cAMP) concentration increase phosphorylation exclusively at Ser$^{40}$ (Haycock, 1990; Waymire et al., 1991). However, in vitro phosphorylation of the cAMP-dependent protein kinase targeted site on TH by additional protein kinases has been demonstrated (Albert et al., 1984; Vulliet et al., 1985; Roskoski et al., 1987; Funakoshi et al., 1991; Sutherland et al., 1993).

The phosphorylation of Ser$^{40}$ has been associated with TH activation. One model proposes that phosphorylation of...
Ser⁴⁰ dissociates tightly bound catecholates (Haavik et al., 1990; Daubner et al., 1992), which keep TH in an inactive state (Ramsey and Fitzpatrick, 1998). Vulliet et al. (1980) showed that cAMP-dependent protein kinase phosphorylates purified TH, concomitantly with increases in TH activity. With either forskolin or high-potassium treatment of cells expressing recombinant TH (rTH), increases in Ser⁴⁰ phosphorylation correlate with increases in DOPA biosynthesis (Harada et al., 1996). In contrast, a direct involvement of Ser¹⁹ phosphorylation in TH activation in vitro seems unlikely (Sutherland et al., 1993). Moreover, in cells expressing rTH with a Ser¹⁹ mutation, there is little difference in the mutant TH compared with wild-type rTH transfected cells (Haycock et al., 1998). However, Ser³¹ phosphorylation may regulate TH activity. For example, high K⁺-induced increases in catecholamine (CA) biosynthesis occur in cells expressing rTH with a Ser⁴⁰ mutation (Harada et al., 1996). In addition, increases in TH activity are associated with its phosphorylation by extracellular signal-regulated protein kinase in vitro (Haycock et al., 1992; Sutherland et al., 1993).

In the CNS, medial forebrain bundle stimulation increases striatal TH activity (Murrin et al., 1976), dopamine (DA) biosynthesis (Murrin and Roth, 1976), and ³²P incorporation at Ser¹⁹, Ser³¹, and Ser⁴⁰ in striatal TH (Haycock and Haycock, 1991). Because both Ser¹⁹ and Ser⁴⁰ phosphorylation increase with this treatment, it is impossible to distinguish their relative involvement in regulating TH activity or DA biosynthesis in vivo. However, treatment with γ-butyrolactone (GBL) results in an increase in Ser⁴⁰ (and not Ser³¹) phosphorylation that is concomitant with an increase in striatal TH activity and DOPA biosynthesis in vivo (Lew et al., 1998). Nonetheless, the lack of effect of GBL on Ser³¹ phosphorylation does not rule out an association of Ser³¹ phosphorylation with TH activity in vivo, particularly as other studies suggest a relationship thereof to TH activity.

Acute administration of neuroleptics such as haloperidol increases TH activity and CA biosynthesis in the terminal field regions of the nigrostriatal and mesolimbic DA systems (Zivkovic and Guidotti, 1974; Zivkovic et al., 1975b; Nissbrandt et al., 1989). In contrast, the effects of haloperidol in the cognate cell body regions are much smaller (Zivkovic et al., 1974; Westerink and Korf, 1976; Argiolas et al., 1979, 1980, 1982; Nissbrandt et al., 1989). To the extent that such compartmental differences in the effects of haloperidol may reflect differences in TH phosphorylation stoichiometry (PS), we used affinity-purified phosphorylation-state specific antibodies and calibrated phosphorylated TH standards to measure TH PS in the terminal field and cell body regions of the nigrostriatal and mesolimbic DA systems.

**MATERIALS AND METHODS**

**Animals and drugs**
Male Harlan Sprague–Dawley rats (230–310 g) were used. The animals were kept four per cage under controlled environmental conditions (22°C, 12:12-h light/dark cycle, food and water available ad libitum) for at least 5 days prior to experiment. Animals were housed according to drug treatment. Haloperidol, SCH23390, raclopride, and clozapine were purchased from Research Biochemicals International. Drugs were dissolved in glacial acetic acid and diluted with NaOH to a final pH range of 5.0–6.5. Vehicle was a sodium acetate solution of comparable concentration and pH.

**Treatment and dissection**
All drug and vehicle treatments were administered intraperitoneally. Treated animals were returned to the same cage and room in which they were housed. In a separate room, animals were decapitated 30–40 min after injection. The brains were immediately removed and partially dissected in 1-mm coronal slices using a chilled rodent brain dissection apparatus. The striatum (Str) and nucleus accumbens (NAc) were punched from one coronal slice with a 13-gauge needle. The substantia nigra (SN) and ventral tegmental area (VTA) were separated free-hand from a coronal slice using a scalpel blade. The entire hypothalamus (Hyp) was then dissected from the intervening brain tissue. Harvested tissue was immediately frozen on dry ice in microfuge tubes and kept at −70°C until processing for protein assay and blot immunolabeling.

**Primary antibodies and blot immunolabeling standards**
An affinity-purified rabbit polyclonal antibody to TH (Haycock, 1989) was used to assay total TH (tTH). Site- and phosphorylation state-specific antibodies to Ser¹⁹ and Ser⁴⁰ were generated as previously described (Haycock et al., 1998). Antibodies to phospho-Ser³¹ were generated against the peptide AVTS(PO₃)PRFIGC[Cys³⁶]-TH(28–37) coupled to keyhole limpet hemocyanin via the cysteine residue. Antibodies were affinity-purified by a multiple-step procedure (Haycock et al., 1998) using SulfoLink gel (Pierce) columns to which the phosphopeptide and the cognate nonphosphopeptide were coupled. Criterion testing for selective reactivity of the antibodies with the phospho versus nonphospho form of TH was performed as previously described (Haycock et al., 1998). The specificity of the antibody to phospho-Ser³¹ has been previously demonstrated (Lew et al., 1998).

Standards used to determine site-specific TH PS were calibrated based upon immunoreactivity with the appropriate phosphorylation state-specific antibody and, for Ser¹⁹ and Ser⁴⁰, upon immunoreactivity with the appropriate non-phosphorylation state-specific antibody. In vitro phosphorylated and nonphosphorylated TH preparations were used as appropriate in the calibration of the standards (Haycock et al., 1998). Calibration of Ser³¹ PS standards was estimated using a value of 0.6 for Ser³¹ PS in PC12 cells treated for 40–60 min with 2 μM okadaic acid. Based upon this value, the relative TH PS among the three TH phosphorylation sites in Str of vehicle-injected controls (VICs) was identical to that of ³²P incorporation reported previously (Haycock and Haycock, 1991).

**Blot immunolabeling**
Frozen tissue samples were sonicated in 1% sodium dodecyl sulfate solution (pH ~8) using a Kontes Microultrasonic Cell Disruptor for ~10 s. Protein concentration was determined for each sample using the bicinchoninic acid method (Smith et al., 1985). Reducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis sample buffer was then added to each sample to equalize total protein across all samples in each brain region. Quantitative blot immunolabeling was done as described by...
The amount of tTH, relative to total protein, in each brain region assayed in this study is presented in Table 1. The VTA had twofold or greater tTH per total protein than any other brain region. The comparatively lower tTH amount in SN was likely due to the inclusion of the pars reticulata component of the SN during dissection.

TH PS in VIC

The overall means of TH PS for Ser<sup>19</sup>, Ser<sup>31</sup>, and Ser<sup>40</sup> in the VIC group are presented in Fig. 1. The terminal field regions had a two- to fourfold higher level of Ser<sup>31</sup> PS than the cognate cell body regions. Conversely, the terminal field regions had a two- to fourfold higher level of Ser<sup>31</sup> PS than the cognate cell body regions. Ser<sup>40</sup> PS was the lowest TH PS value in all regions and was comparable in the terminal field and cell body regions of both DA systems.

Ser<sup>40</sup> PS in hypothalamus was 0.049 ± 0.003 (n = 3; mean ± SEM). Whereas this particular TH PS value was greater than those in the DA regions, Ser<sup>19</sup> and Ser<sup>31</sup> PS (0.26 ± 0.02 and 0.13 ± 0.01, respectively) were higher than Ser<sup>40</sup> PS, similar to each DA region.

**Effects of haloperidol on TH phosphorylation**

The effects of haloperidol on each TH phosphorylation site in each DA region are illustrated in the blot immunolabeling profile in Fig. 2. The quantitative data have been summarized in Table 2 and Fig. 3. After haloperidol treatment, roughly twofold increases in TH PS at Ser<sup>19</sup>, Ser<sup>31</sup>, and Ser<sup>40</sup> were observed in the terminal field regions (Str and NAc) (Table 2; Figs. 2 and 3). The effects of haloperidol in the cell body regions differed from those in the terminal fields in that increases in TH PS comparable with those seen in a terminal field region (Str) occurred only at Ser<sup>31</sup> (Fig. 3B). There was no significant difference in the increase in Ser<sup>31</sup> PS after haloperidol between Str, SN, or VTA (Table 2). However, the increase in Ser<sup>40</sup> PS in the SN was significantly less than that in Str (Fig. 3C). The increase in Ser<sup>40</sup> PS in the VTA was also significantly less than that in NAc (Fig. 3C). The difference in the increase in Ser<sup>40</sup> PS between SN and VTA was not statistically significant (Table 2). However, seven of seven SN samples from haloperidol-treated rats had greater Ser<sup>40</sup> PS than the operationally matched VIC rats. In the animal-matched VTA samples, only five of the seven haloperidol-treated rats had greater Ser<sup>40</sup> PS than the matched VIC rats. In addition, the average increase in Ser<sup>40</sup> PS in these five samples was half of that in the corresponding SN samples. Significant differences in the increase in Ser<sup>19</sup> PS after haloperidol were also seen between the terminal field and cell body regions (Fig. 3A).

**RESULTS**

**tTH in nigrostriatal system, mesolimbic system, and Hyp**

The amount of tTH, relative to total protein, in each brain region assayed in this study is presented in Table 1. The VTA had twofold or greater tTH per total protein than any other brain region. The comparatively lower tTH amount in SN was likely due to the inclusion of the pars reticulata component of the SN during dissection.

**Statistical analysis**

Drug treatment effects on TH PS were determined by dividing the treatment-associated TH PS value by the operationally matched VIC TH PS value. A percentage of control value was obtained. A repeated-measures ANOVA test was applied to determine if there was a significant difference in the percent change in TH PS among Str, NAc, SN, and VTA samples associated with each drug treatment at each phosphorylation site (the phosphorylation sites were assumed to be independent). If a significant difference (p < 0.05) was detected, a direct comparison of brain regions was made using the least significant difference method (p < 0.05).

**TABLE 1. tTH protein levels in nigrostriatal and mesolimbic systems and in Hyp**

<table>
<thead>
<tr>
<th>Region</th>
<th>n</th>
<th>tTH (ng/μg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Str</td>
<td>32</td>
<td>0.42 ± 0.05</td>
</tr>
<tr>
<td>SN</td>
<td>32</td>
<td>0.27 ± 0.06</td>
</tr>
<tr>
<td>NAc</td>
<td>32</td>
<td>0.28 ± 0.03</td>
</tr>
<tr>
<td>VTA</td>
<td>32</td>
<td>1.00 ± 0.22</td>
</tr>
<tr>
<td>Hyp</td>
<td>12</td>
<td>0.07 ± 0.01</td>
</tr>
</tbody>
</table>

The TH PS values are means ± SEM. The number of analyses representing each region exceeds the total number of animals (24) used in the TH PS analysis. tTH data from a separate pilot study are also included.
The effect of haloperidol treatment on TH PS in Hyp is summarized in Fig. 4. The mean ± SEM increases (as percentage of control) were 120 ± 9% for Ser19, 112 ± 6% for Ser31, and 113 ± 10% for Ser40.

**Effects of raclopride, clozapine, and SCH23390 on TH phosphorylation**

The effects of raclopride on TH PS in each DA region were similar to those of haloperidol. In the terminal field regions, twofold increases in Ser19 and Ser40 PS were observed (Table 2; Fig. 3A and C). In the cell body regions, increases in TH PS similar to those in a terminal field region were observed.

<table>
<thead>
<tr>
<th>Region</th>
<th>Treatment</th>
<th>Ser19</th>
<th>Ser31</th>
<th>Ser40</th>
</tr>
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<tbody>
<tr>
<td>Str</td>
<td>Haloperidol</td>
<td>248 ± 46</td>
<td>173 ± 14</td>
<td>235 ± 15</td>
</tr>
<tr>
<td></td>
<td>Raclopride</td>
<td>224 ± 45</td>
<td>186 ± 6</td>
<td>234 ± 23</td>
</tr>
<tr>
<td></td>
<td>Clozapine</td>
<td>186 ± 50</td>
<td>128 ± 12</td>
<td>235 ± 33</td>
</tr>
<tr>
<td></td>
<td>SCH23390</td>
<td>93 ± 10</td>
<td>93 ± 4</td>
<td>117 ± 9</td>
</tr>
<tr>
<td>SN</td>
<td>Haloperidol</td>
<td>121 ± 6</td>
<td>160 ± 12</td>
<td>130 ± 11</td>
</tr>
<tr>
<td></td>
<td>Raclopride</td>
<td>103 ± 7</td>
<td>172 ± 24</td>
<td>112 ± 4</td>
</tr>
<tr>
<td></td>
<td>Clozapine</td>
<td>128 ± 12</td>
<td>200 ± 17</td>
<td>133 ± 4</td>
</tr>
<tr>
<td></td>
<td>SCH23390</td>
<td>87 ± 7</td>
<td>100 ± 17</td>
<td>90 ± 14</td>
</tr>
<tr>
<td>NAc</td>
<td>Haloperidol</td>
<td>205 ± 22</td>
<td>267 ± 25</td>
<td>236 ± 21</td>
</tr>
<tr>
<td></td>
<td>Raclopride</td>
<td>203 ± 26</td>
<td>279 ± 23</td>
<td>205 ± 16</td>
</tr>
<tr>
<td></td>
<td>Clozapine</td>
<td>207 ± 32</td>
<td>244 ± 30</td>
<td>248 ± 34</td>
</tr>
<tr>
<td></td>
<td>SCH23390</td>
<td>154 ± 16</td>
<td>154 ± 15</td>
<td>123 ± 8</td>
</tr>
<tr>
<td>VTA</td>
<td>Haloperidol</td>
<td>109 ± 9</td>
<td>160 ± 12</td>
<td>111 ± 5</td>
</tr>
<tr>
<td></td>
<td>Raclopride</td>
<td>117 ± 13</td>
<td>168 ± 27</td>
<td>98 ± 4</td>
</tr>
<tr>
<td></td>
<td>Clozapine</td>
<td>147 ± 12</td>
<td>233 ± 7</td>
<td>135 ± 6</td>
</tr>
<tr>
<td></td>
<td>SCH23390</td>
<td>104 ± 29</td>
<td>147 ± 35</td>
<td>98 ± 6</td>
</tr>
</tbody>
</table>

TH PS values are presented as percentage of VIC (means ± SEM) for each drug treatment in the four dopaminergic regions. Each region was removed 30–40 min following intraperitoneal injection of each drug at the following dosages (haloperidol, 2 mg/kg; raclopride, 2 mg/kg; SCH23390, 0.5 mg/kg; clozapine, 30 mg/kg). Collected tissue was immediately frozen on dry ice and kept at −70°C until processing for blot immunolabeling as described. Samples were assayed with calibrated TH PS standards for TH PS at each phosphorylation site in a manner that included all drug treatments in each of three analyzed animal groups. Total number of animals per treatment was as follows: haloperidol (n = 7), raclopride (n = 4), SCH23390 (n = 3), clozapine (n = 3).
field region (Str) occurred only at Ser\(^{31}\) after raclopride (Table 2; Fig. 3B). However, there was a significant difference in the increase in Ser\(^{40}\) PS in the Str compared with that in SN (Fig. 3C). There was no increase in Ser\(^{40}\) PS in the VTA, whereas a twofold increase in Ser\(^{40}\) PS occurred in NAc (Table 2; Fig. 3C) after raclopride. In addition, there was a significant difference in the increase in Ser\(^{19}\) PS in both terminal field regions compared with their cognate cell body regions (Fig. 3A).

After clozapine treatment, TH PS increased at all three phosphorylation sites in each region. Ser\(^{40}\) PS and Ser\(^{19}\) PS were increased to a similar extent in the cell body regions (Table 2). However, there was a significant difference in these increases in the cell body regions compared with their cognate terminal field regions (Fig. 3A and C). The increase in Ser\(^{19}\) PS was greater than that of Ser\(^{40}\) or Ser\(^{19}\) in the cell bodies and was not significantly different from the increase in NAc (Table 2). However, there was a significant difference in the increase in Ser\(^{31}\) PS between Str and NAc after clozapine treatment (Fig. 3B). There was, however, no significant difference in the increase of Ser\(^{40}\) PS or Ser\(^{19}\) PS between Str and NAc after clozapine treatment (Table 2; Fig. 3A and C).

In Hyp, there was an increase in TH PS at all three phosphorylation sites after clozapine treatment. These increases were larger than those produced by haloperidol (Fig. 4).

There was no significant difference in TH PS changes at any phosphorylation site between Str, NAc, SN, and VTA after SCH23390 treatment (Table 2; Fig. 3). However, the greatest increases in TH PS occurred in the NAc at each phosphorylation site (Table 2). In Str, an increase in Ser\(^{40}\) PS was similar to that observed in NAc (Table 2; Fig. 3C).

**DISCUSSION**

The PS of TH is reported for the first time in the nigrostriatal and mesolimbic DA systems. In the VIC group, these systems share similar features of TH PS in that Ser\(^{19}\) PS and Ser\(^{31}\) PS are dissimilar between the terminal field and cell body regions (Fig. 1). Thus, a common feature between the two DA systems is that Ser\(^{19}\) PS is lower and Ser\(^{31}\) PS is greater in both the Str and the NAc than in the SN and VTA. Conversely, Ser\(^{40}\) PS is similar between these neuronal compartments in either DA system and has the lowest value. Thus, the compartmental differences in Ser\(^{19}\) and Ser\(^{31}\) TH PS are a common feature of the nigrostriatal and mesolimbic systems. However, Ser\(^{40}\) PS is similar between the neuronal compartments in both systems and is also the lowest TH PS value in all four regions.

In a previous study of in vivo TH phosphorylation in striatum, \(^{32}\)P incorporation among the three sites in non-treated rats was highest at Ser\(^{19}\), followed by Ser\(^{19}\) and then Ser\(^{40}\) (Haycock and Haycock, 1991). We found striatal TH PS levels to be greatest at Ser\(^{31}\) (\(-0.3\)), followed by Ser\(^{19}\) (0.10) and then Ser\(^{40}\) (0.03) in the VIC group. Many studies have been devoted to studying the relationship between TH phosphorylation and activity in situ. However, whether or not TH PS in situ reflected actual activity in vivo levels was not known. This first in vivo TH PS study shows that TH PS values in situ are very similar to those in vivo (Haycock et al., 1998; unpublished observations).

In the VIC group, Ser\(^{40}\) PS was \(-0.03\) in either DA system. This value is anywhere from three- to 10-fold lower than the TH PS of Ser\(^{19}\) or Ser\(^{31}\) among the four regions (Fig. 1). Ser\(^{40}\) PS increases roughly twofold in the terminal field regions after acute haloperidol, a DA D\(_2\) antagonist, or clozapine. These drugs have been previously shown to increase TH activity and CA biosynthesis (Zivkovic et al., 1974, 1975; Magnusson et al., 1987; Nissbrandt et al., 1989). However, in spite of this twofold increase, actual Ser\(^{40}\) PS is still \(<0.1\). Similarly, increases in Ser\(^{40}\) PS from elevated extracellular potassium treatment, which are associated with increases in CA biosynthesis in situ, have also \(<0.1\) stoichiometry (Haycock et al., 1998). Thus, it appears that a twofold increase in vivo, even though Ser\(^{40}\) PS still is \(<0.1\), may be sufficient to increase TH activity and CA biosynthesis. Therefore, if Ser\(^{40}\) phosphorylation regulates TH activity in vivo, this relatively low Ser\(^{40}\) PS value suggests that TH activity is kept at a minimum in the CNS and comparatively small changes in phosphorylation are necessary for TH activation.

An association of Ser\(^{40}\) phosphorylation with TH activity and CA biosynthesis in vivo has recently been shown. Striatal Ser\(^{40}\) TH phosphorylation was increased twofold after GBL treatment, concomitantly with increases in TH activity and DOPA biosynthesis (Lew et al., 1998). By contrast, Ser\(^{19}\) and Ser\(^{31}\) phosphorylation were relatively unaffected. However, TH activation has been shown to occur in the absence of Ser\(^{40}\) phosphorylation, because elevated extracellular potassium increases DOPA biosynthesis to the same extent in transfected cells expressing either wild-type rTH or S40L rTH (Harada et al., 1996). In addition, whereas Ser\(^{40}\) phosphorylation increases in vivo after medial forebrain bundle stimulation adequate to increase DOPA biosynthesis, phosphorylation also increases at Ser\(^{31}\) and Ser\(^{19}\) (Haycock and Haycock, 1991). Therefore, the lack of an
increase in Ser\(^{31}\) phosphorylation following GBL does not necessarily rule out that Ser\(^{31}\) phosphorylation could influence TH activity in vivo, at least with other TH-activating treatments.

DA D\(_2\) receptor antagonists such as haloperidol increase TH activity and CA biosynthesis in the nigrostriatal system. However, the increases in SN are less than those in the Str (Argiolas et al., 1982; Magnusson et al., 1987; Nissbrandt et al., 1989). Therefore, these drugs may also produce dissimilar changes in TH PS in these regions. Indeed, the increases in TH PS after haloperidol or raclopride treatment differed between Str and SN. The increase in Ser\(^{40}\) PS in SN after haloperidol was about one-fourth of that in Str (Fig. 3C). However, there was no significant difference in the increase in Ser\(^{31}\) PS between these two regions (Fig. 3B). These data suggest that increases in Ser\(^{31}\) phosphorylation are not correlative with increases in CA biosynthesis in vivo. However, the difference in the increase in Ser\(^{40}\) PS between SN and Str seems to be correlative with previously shown compartmental differences in CA biosynthesis after these drug treatments and suggests that Ser\(^{40}\) phosphorylation is associated with TH activity in the nigrostriatal system.

Haloperidol and DA D\(_2\) receptor antagonists produce similar differences in CA biosynthesis between the terminal field and cell body region in the mesolimbic system. Haloperidol treatment increases the DA turnover rate in NAc (Zivkovic et al., 1975b). However, acute haloperidol treatment does not increase 3,4-dihydroxyphenylacetic acid levels in VTA (Argiolas et al., 1979, 1980). These data suggest that there would be little, if any, effect on Ser\(^{40}\) PS in the VTA after haloperidol or raclopride. In fact, whereas Ser\(^{40}\) PS increased twofold in NAc with either haloperidol or raclopride, haloperidol produced an increase in Ser\(^{40}\) PS in only five of seven VIC-matched VTA samples. Raclopride did not increase Ser\(^{40}\) PS in any of the four VIC-matched samples (Table 2). However, both haloperidol and raclopride increased Ser\(^{31}\) PS in the VTA to an extent similar to that in SN and Str (Fig. 3B). The increases in Ser\(^{31}\) PS following haloperidol do not reflect the compartmental differences in CA biosynthesis previously shown in both the nigrostriatal and the mesolimbic system. However, the increases in Ser\(^{40}\) PS reflect the previously shown compartmental differences in CA biosynthesis in both systems.

Haloperidol has little effect on TH activity or CA biosynthesis in the hypothalamus (Zivkovic et al., 1974; Magnusson et al., 1987). Clozapine, however, has been shown to increase DOPA accumulation in Hyp (Gudelsky and Meltzer, 1989). Consistent with these observations, clozapine treatment produced greater increases in TH PS (including Ser\(^{40}\)) than haloperidol treatment (Fig. 4).

Like haloperidol, clozapine has been shown to increase DA turnover in both Str and NAc (Zivkovic et al., 1975b). Clozapine treatment increased Ser\(^{40}\) PS at least twofold in both Str and NAc. Similar increases in Ser\(^{19}\) PS occurred after haloperidol treatment (Fig. 3C). However, clozapine treatment increased Ser\(^{31}\) PS significantly less in Str than in NAc (Fig. 3B). This difference in Ser\(^{31}\) PS, and not Ser\(^{40}\), may also suggest that Ser\(^{31}\) is not directly involved with regulating CA biosynthesis through TH phosphorylation.

Unlike clozapine or haloperidol, SCH23390 treatment has been shown to have only small effects on CA biosynthesis in the terminal regions (Iorio et al., 1983; Hjorth and Carlsson, 1988). Although SCH23390 treatment did not produce any significant differences in TH PS among the four regions, TH PS increased at all sites in the NAc. It is perhaps consistent with previous studies, however, that in both Str and NAc, SCH23390 treatment increased Ser\(^{40}\) PS to roughly 120% of control (Table 2; Fig. 3C).

The in vivo TH PS data, in addition to showing relationships to compartmental differences in CA biosynthesis in the nigrostriatal and mesolimbic system, may also have implications in understanding the mechanism of action of neuroleptic drugs in vivo. There are conflicting schools of thought as to how dopaminergic drugs affect striatal TH activity. One hypothesis is that DA D\(_2\) drugs like haloperidol act on postsynaptic DA receptors associated with the striatopallidodionigral loop, which governs impulse flow to the nigrostriatal neuron (Zivkovic et al., 1975a; Gale et al., 1978). Upon binding to the postsynaptic receptor, there is a reduction in tonic inhibitory GABAergic input to the nigrostriatal neuron, which results in an increase in nigrostriatal neuron activity that in turn activates TH. Conversely, TH activity may be controlled via presynaptic DA D\(_2\)-like autoreceptors through modulation of cAMP-dependent signal transduction (Strait and Kuczenski, 1986; Salah et al., 1989). Antagonist blockade of these receptors presumably prevents released DA from binding these receptors, an action that presumably decreases TH activity via down-regulation of cAMP-dependent signal transduction. However, one contradiction to the presynaptic hypothesis is that direct application of neuroleptics to Str or NAc homogenates does not alter TH activity (Zivkovic and Guidotti, 1974; Zivkovic et al., 1975b).

The effect of haloperidol on striatal TH PS seen in this study is similar to that of electrical stimulation of the nigrostriatal pathway, because TH phosphorylation increases at all three sites after such stimulation (Haycock and Haycock, 1991). This overall increase in TH PS in Str may be reflective of an increase in depolarization of the nigrostriatal neuron, which has been associated with acute haloperidol administration (Bunney et al., 1973, 1980). In accordance with the postsynaptic mechanism, the overall increase in TH PS suggests that haloperidol is acting through postsynaptic DA receptors. If the in vivo effects of haloperidol on TH PS were mediated solely by autoreceptors, one would expect that only Ser\(^{40}\) PS would increase; haloperidol occupancy of a presynaptic DA D\(_2\) receptor would presumably prevent down-regulation of cAMP-dependent signal transduction in rat neostriatum because the DA D\(_2\) receptor is negatively linked to cAMP-dependent signal transduction (Stoof
and Kebabian, 1981). However, both Ser\(^{19}\) and Ser\(^{31}\) PS increase after haloperidol or raclopride treatment. The phosphorylation of either site is relatively unaffected by cAMP-dependent signal transduction (Haycock, 1990). There is, however, some indication that autoreceptors may also be involved. The increases in Ser\(^{40}\) PS caused by these drugs differed between the terminal field and cell body regions and to a lesser extent between SN and VTA. These differences in Ser\(^{40}\) PS may be related to regional differences in presynaptic receptor regulation of CA release. Evidence suggests that the DA D\(_2\) receptor exerts greater control of evoked DA release in the Str than in SN (Cragg and Greenfield, 1997; Hoffman and Gerhardt, 1999). Thus, assuming that the autoreceptor is linked to cAMP-dependent signal transduction, the effect of haloperidol on TH PS in vivo may involve both models of TH regulation. The first process is postsynaptic receptor activation of the striatopallidal/loop to increase nigrostriatal neuron activity by increased impulse flow. As a result, TH PS increases at all sites. However, the released DA from this stimulation may influence cAMP-dependent signal transduction (and thus Ser\(^{40}\) PS) differentially between Str and SN, given the differential influence of release-modulating autoreceptors in these regions presumably occupied by haloperidol. The differential effect of haloperidol and raclopride on Ser\(^{40}\) and not Ser\(^{31}\) PS (both of which increase by depolarization in vivo) between these regions could be explained by considering both a postsynaptic and a presynaptic action of haloperidol.

In summary, TH PS is similar between the nigrostriatal and mesolimbic systems in that there are similar differences in PS at Ser\(^{19}\) and Ser\(^{31}\) between the terminal field and cell body regions: Ser\(^{19}\) PS is greater in the cell bodies and Ser\(^{31}\) PS is greater in the terminal fields. However, there is little, if any, difference in Ser\(^{40}\) PS between the terminal field and cell body regions in either DA system. Furthermore, the level of PS at Ser\(^{40}\) is three- to 10-fold lower than Ser\(^{19}\) or Ser\(^{31}\) PS in any region. This low level of Ser\(^{40}\) PS suggests that TH activity is kept at a minimum in vivo. The effects of haloperidol and related compounds on Ser\(^{40}\) PS, but not Ser\(^{31}\) PS, in each DA region bear a remarkable similarity to the previously shown effects of these compounds on TH activity and CA biosynthesis. Finally, the TH PS data suggest that the in vivo mechanism of haloperidol action on TH in the nigrostriatal system involves both postsynaptic and presynaptic DA receptors. The knowledge of TH PS in vivo may be applied to understanding the neurochemistry and physiology of CA function in the CNS.

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