APOMORPHINE- AND HALOPERIDOL-INDUCED CHANGE IN 3,4-DIHYDROXYPHENYLACETIC ACID CONTENT IN THE MESOLIMBIC-STRIATUM OF THE DEVELOPING RAT

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The mesolimbic-striatal content of 3,4-dihydroxyphenylacetic acid (DOPAC) gradually increased with age in the developing rat. An intraperitoneal (i.p.) injection of apomorphine (2 mg/kg) or haloperidol (0.1 mg/kg) caused a significant change in the DOPAC content on day 20 and day 70, but not on day 7. However, a higher dose of apomorphine (10 mg/kg, i.p.) or haloperidol (5 mg/kg, i.p.) as well as that of α-flupenthixol (0.5 mg/kg, i.p.) significantly affected the mesolimbic-striatal content of DOPAC in 7-day-old rats. In 24-day-old rats subchronically treated with haloperidol (1 mg/kg to 10 mg/kg for 10 days, s.c.), apomorphine (2 mg/kg, i.p.) produced a significant reduction in the mesolimbic content of DOPAC at the withdrawal stage of the drug, but not in control rats. It is suggested that mesolimbic-striatal dopamine (DA) receptors which are not fully sensitive to DA agonists and antagonists on day 7 reach functional maturity by 20 days of the postnatal age in the rat.

Keywords — development; DOPAC; mesolimbic-striatum; apomorphine; haloperidol

INTRODUCTION

It has been observed that amphetamine or methamphetamine induces locomotor stimulation in 10-day-old rats or in 1- to 3-day-old rats (Nomura et al., unpublished observations). Spiroperidol, a dopamine (DA) receptor antagonist, causes catalepsy in rats aged 1, 5 and 10 days. In addition, the specific binding of [3H]haloperidol in striatal membranes, which is between 10 and 15% of adult levels at birth shows little change until 7 days and followed by a rapid increase. From a fact that the subchronic treatment with haloperidol in developing rats significantly enhances the apomorphine-induced reduction of the striatal content of homovanillic acid (HVA) at the drug-free period on day 24 and day 34, but not on day 14, it is presumed that a functional link in the feedback regulation mechanisms of DA neuronal activity and DA receptors in the striatum does not fully develop at birth until after around 20 days. However, the ontogenesis in the feedback regulation mechanism on DA neuronal activity is not known in the mesolimbic system.

To gain further insight into the ontogenic development of DA receptor sensitivity in regions of the striatum and the mesolimbic system, we investigated the effects of apomorphine and haloperidol on 3,4-dihydroxyphenylacetic acid (DOPAC) content in the mesolimbic-striatum of 7-day-old, 20-day-old and 70-day-old rats. Furthermore, in the present paper, we studied the effect of apomorphine on the mesolimbic contents of HVA and DOPAC at the withdrawal stage of the drug in the developing rat subchronically treated with haloperidol.

MATERIALS AND METHODS

Albino rats (Wistar) of both sexes were used. After birth all litters were culled to 8 to 10 pups/mother. Pups were maintained with their
mother at 23° at normal daylight conditions, and weaned at 22 days.

In order to examine the effects of haloperidol (0.1 mg/kg and 5 mg/kg), α-flupenthixol (0.1 mg/kg and 0.5 mg/kg) and apomorphine (2 mg/kg and 10 mg/kg) on the mesolimbic-striatal content in the developing rat, all animals were injected intraperitoneally (i.p.) with these drugs and subsequently sacrificed by decapitation at the following intervals after treatment with various drugs: haloperidol (120 min); α-flupenthixol (120 min); apomorphine (30 min). These intervals were chosen after considering both the onset and the duration of action of the drug.

In the experiments of subchronic treatment with haloperidol, a freshly prepared drug solution was administered by subcutaneous (s.c.) injection to the animals once daily, beginning with a dose of 1 mg/kg. The dosage was increased by 1 mg/kg every day until a final dose of 10 mg/kg was reached. The treatment was carried out in 2 different stages in developing rats as follows: 1) 11 to 20 days after birth (HALOP 11-20); 2) 21 to 30 days after birth (HALOP 21-30). The control rats were treated with the vehicle according to the same schedule as described above. Animals on day 24 or day 34 (4 days after the last injection of the drug) were sacrificed by decapitation at 30 min after an i.p. injection of apomorphine (2 mg/kg).

The brain was rapidly removed and dissected over ice. Dissection was carried out by the method of Nomura et al. (1979). After opening the lateral ventricle, the striatum was dissected. An area including the nucleus accumbens and the tuberculum olfactorium as major components was designated as the mesolimbic region. The mesolimbic-striatal tissue contained both striatal and mesolimbic regions. The mesolimbic-striatal or the mesolimbic tissue from 3 rats (day 7) or 2 rats (day 20, day 24, day 34 and day 70) was homogenized in 0.1 N HCl for HVA and DOPAC assays.

HVA and DOPAC were estimated spectrophotofluorimetrically by the method of Murphy et al. (1969). The recoveries of HVA and DOPAC were: HVA: 66.6±9.1% (mean ± S. E., n=8) and DOPAC: 73.8±7.6% (mean ± S. E., n=8); all values were corrected for these recoveries.

Differences between experimental values were analyzed by Student's t-test.

One to 10 mg of haloperidol was dissolved in 1 ml of propylene glycol: ethylalcohol: H2O:0.1 N HCl (20:5:25:13, v/v), and the mixture made up with saline to 10 ml. α-Flupenthixol was dissolved in saline and apomorphine in saline containing 0.001 N HCl. Preliminary experiments showed that neither vehicle of haloperidol nor that of apomorphine affected the mesolimbic-striatal DOPAC and HVA contents.

Haloperidol was generously supplied from Dainippon Pharmaceutical Co., Ltd., Osaka, Japan. α-Flupenthixol hydrochloride and apomorphine hydrochloride from Takeda Chemical Industries, Ltd., Osaka, Japan. Homovanillic acid and 3,4-dihydroxyphenylacetic acid were purchased from Nakarai chemicals Co., Ltd., Kyoto, Japan.

RESULTS

1) The Effects of Haloperidol and Apomorphine on the Mesolimbic-striatal Content of DOPAC in Developing Rats

Fig. 1 shows the effect of an i.p. administration of haloperidol (0.1 mg/kg) or apomorphine (2.0 mg/kg) on the mesolimbic-striatal content of DOPAC in developing rats. Haloperidol significantly increased the DOPAC content by 78.2% (p<0.001) in 20-day-old rats and by 52.7% (p<0.001) in 70-day-old rats. An injection of apomorphine produced a significant reduction in the mesolimbic-striatal content by 21.2% (p<0.01) in 20-day-old rats and by 45.7% (p<0.001) in 70-day-old animals. By contrast, neither haloperidol (0.1 mg/kg, i.p.) nor apomorphine (2 mg/kg, i.p.) caused a change in the mesolimbic-striatal content of DOPAC in 7-day-old rats.

2) The Effects of Haloperidol, α-Flupenthixol and Apomorphine on the Mesolimbic-striatal Content of DOPAC in 7-day-old Rats

Although a low dose of haloperidol (0.1 mg/kg) or apomorphine (2.0 mg/kg) did not
induce a significant change in the mesolimbic-striatal DOPAC content of newborn rats (Fig. 1), an i.p. administration of haloperidol (5.0 mg/kg) or apomorphine (10.0 mg/kg) resulted in a significant change (p < 0.05) in the DOPAC content as shown in Fig. 2. Furthermore, α-flupenthixol did not induce a significant change at a dose of 0.1 mg/kg (i.p.) but did induce a significant increase at a higher dose of 0.5 mg/kg (i.p.).

3) The Effect of Apomorphine on the Mesolimbic Contents of HVA and DOPAC in developing Rats subchronically treated with Haloperidol

A subchronic treatment with haloperidol for 10 days induced the significant decrease (p < 0.05) in the mesolimbic HVA content, but not in the DOPAC content at the drug-free period (day 24 in HALOP 11-20 and day 34 in HALOP 21-30) (Fig. 3). In 24-day-old rats, subchronic treatment with haloperidol enhanced the apomorphine (2.0 mg/kg, i.p.)-induced reduction of the mesolimbic content of HVA compared to control (Fig. 3 and Table 1). On day 34, apomorphine (2.0 mg/kg, i.p.) produced a significant reduction in the HVA content in both control and haloperidol-pre-treated rats. In addition, the mesolimbic DOPAC content was reduced by an i.p. injection of apomorphine (2.0 mg/kg) in control and haloperidol-treated animals aged 24 and 34 days.

**DISCUSSION**

The mesolimbic-striatal content of DOPAC in adult rats was 3.3 times that of 7-day-old animals. The content increased with age more steeply than that in the whole brain reported by Keller et al. (1973). The developmental change of DOPAC as well as DA content in the limbic-striatum suggests that the innervation and neuronal activity of DA neurons in the striatum and the mesolimbic region gradually proceed during the neonatal period in the rat.

Neither haloperidol (0.1 mg/kg) nor apomorphine (2.0 mg/kg) significantly affected the mesolimbic-striatal content of DOPAC in 7-day-old rats, while both drugs in the same doses

**FIG. 1. The Effects of Haloperidol and Apomorphine on the Mesolimbic-striatal Content of DOPAC in developing Rats**

Animals were sacrificed at 120 min (haloperidol) and 30 min (apomorphine) after an i.p. injection of haloperidol (0.1 mg/kg) or apomorphine (2.0 mg/kg) and the DOPAC content was determined. The DOPAC content is expressed as mean value with S.E. of 3–5 determinations. ○ --- ○, control; ■ --- ■, Halop. (haloperidol); • --- •, Apomor. (apomorphine). **Significant at p < 0.01 vs. control. ***Significant at p < 0.001 vs. control.

**FIG. 2. The Effects of Haloperidol, α-Flupenthixol and Apomorphine on the Mesolimbic-striatal Content of DOPAC in 7-day-old Rats**

Animals were sacrificed and the DOPAC content was estimated at the following intervals after an i.p. injection of drugs: haloperidol (120 min); α-flupenthixol (120 min); apomorphine (30 min). The DOPAC content is expressed as mean value with S.E. of 3–8 determinations. Halop, haloperidol; α-FPT, α-flupenthixol; Apomor., apomorphine. *Significant at p < 0.05 vs. control.
FIG. 3. The Effect of Apomorphine on the Mesolimbic Contents of HVA (upper figure) and DOPAC (lower figure) in developing Rats subchronically treated with Haloperidol.

At 30 min after an injection of apomorphine (2.0 mg/kg), animals were sacrificed and the mesolimbic contents of HVA and DOPAC were estimated. The content is expressed as mean value with S.E. of 4–8 determinations. Apomor, apomorphine; Halop, haloperidol. White column, control; hatched column, apomor- phine 2.0 mg/kg. NS, not significant. * Significant at p < 0.05 vs. control. ** Significant at p < 0.01 vs. control.

TABLE I. Effect of Apomorphine on the Mesolimbic Contents of HVA and DOPAC in the developing Rat subchronically treated with Haloperidol

<table>
<thead>
<tr>
<th>Content of HVA and DOPAC (Per cent of control)</th>
<th>HVA</th>
<th>DOPAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 24</td>
<td>Day 34</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Apomorphine (2 mg/kg)</td>
<td>73.1±9.6</td>
<td>65.9±6.1*</td>
</tr>
<tr>
<td>Chronic haloperidol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Apomorphine (2 mg/kg)</td>
<td>58.8±2.1*</td>
<td>61.5±14.2*</td>
</tr>
</tbody>
</table>

Haloperidol was s.c. injected to the developing rat once daily beginning with a dose of 1 mg/kg. The dosage was increased by 1 mg/kg every day until a final dose of 10 mg/kg was reached. Animals on 4 days after the last injection of the drug were sacrificed and the mesolimbic content of HVA and DOPAC measured. Values are expressed as per cent of the control. * Significant at p < 0.05 vs. control. ** Significant at p < 0.01 vs. control.
induced significant changes in 20- and 70-day-old rats. Although it appears that central DA receptors, which are insensitive to DA agonists and antagonists on day 7, reach functional maturity by 20 days of age, high doses of haloperidol (5 mg/kg) and apomorphine (10 mg/kg) caused a significant change in the DOPAC content on day 7. In addition, α-flupenthixol (0.5 mg/kg), a strong DA receptor antagonist, increased the DOPAC content in 7-day-old rats. Therefore, DA receptors in these regions could be partially responsive to the agonists and the antagonists. This speculation is supported by a finding that there is a little change in the specific [3H]haloperidol binding until 7 days, followed by a rapid increase in the striatum of developing rats. On the other hand, if the change of the DOPAC content induced by haloperidol and apomorphine is an index of sensitivity of the presynaptic DA receptors located on the DA nerve terminals, the present results suggest that an autoregulatory function of presynaptic DA 'autoreceptors' on release and biosynthesis of DA may not fully develop at 7 days, but may reach a functional maturity around 20 days. With regard to the ontogenic development of the HVA content in the rabbit whole brain, Kellogg et al. (1972) demonstrated that haloperidol (0.2 mg/kg, s.c.) caused a significant increase immediately after birth and that the magnitude of the increase was much smaller in newborn than in animals of 3 to 4 weeks after birth.

In contrast to acute experiments of haloperidol, subchronic treatment with haloperidol resulted in a tendency of the reduction of the mesolimbic content of DOPAC and in the significant decrease of HVA content at 4 days after cessation of the antagonist in both HALOP 11–20 and HALOP 21–30 animals. This is in agreement with our previous results concerning the reduction in the striatal contents of DOPAC and HVA in developing rats. An i.p. injection of apomorphine (2 mg/kg) produced a reduction of the HVA and DOPAC contents in control and treated animals on day 24 and day 34; and the long-term treatment with haloperidol enhanced the decrease of HVA content by apomorphine. On the other hand, the subchronic treatment with haloperidol did not significantly change the apomorphine-induced decrease in the DOPAC content. DOPAC, a metabolite of DA by monoamine oxidase (MAO), is mainly of presynaptic origin, while HVA, a metabolite of DA by MAO and catechol O-methyl transferase, is a possible product of DA which is liberated from DA terminals. Thus, the chronic treatment with haloperidol does not seem to change the sensitivity of presynaptic DA receptors, but does the postsynaptic receptors in the mesolimbic region. This also may explain the lack of change in the DOPAC content of repeated injection of haloperidol.

Since apomorphine-induced decrease of the striatal content of HVA is enhanced in rats treated with haloperidol, mesolimbic DA receptors could be modified by the treatment with haloperidol as well as striatal ones at young stage. Inhibition of striatal DA turnover by apomorphine is potentiated in adult rats chronically treated with haloperidol. The specific bindings of [3H]-haloperidol and [3H]apomorphine increased significantly not only in the striatum, but also in the mesolimbic region at 2 days, after a long-term treatment with haloperidol in adult rats. Anatomical study has shown that strong dotted DA fluorescence in nucleus accumbens appears at an earlier postnatal age than diffuse DA fluorescence in the striatum. Thus, it is likely that DA receptors become supersensitive in the striatum and the mesolimbic forebrain after termination of chronic haloperidol in young rats. Behaviorally we observed that the subchronic treatment with haloperidol significantly potentiated apomorphine (0.2 mg/kg, s.c.)-induced locomotor stimulation in the 24-day-old rat at the withdrawal stage of haloperidol (Nomura and Segawa, unpublished observations).

The clarification of regional differences in ontogenic development of DA receptor sensitivity remains to be a future subject.

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