Short Communication

ENDOMORPHINS, Met-ENKEPHALIN, Tyr-MIF-1, AND THE P-GLYCOPROTEIN EFFLUX SYSTEM

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ABSTRACT:
The P-glycoprotein (P-gp) transport system, responsible for the efflux of many therapeutic drugs out of the brain, recently has been shown to transport the endogenous brain opiate endorphin. We used P-gp knockout mice (Mdr1a) and their controls to determine where P-gp is involved in the saturable efflux systems of four other endogenous opiate-modulating peptides across the blood-brain barrier (BBB). After injection of endomorphin-1 (Tyr-Pro-Trp-Phe-NH₂), endomorphin-2 (Tyr-Pro-Phe-Phe-NH₂), Met-enkephalin (Tyr-Gly-Gly-Phe-Met-OH), and Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH₂) into the lateral ventricle of the mouse brain, residual radioactivity was measured at 0, 2, 5, 10, and 20 min later. The results showed no difference in the disappearance of any of these peptides from the brains of knockout mice compared with their controls. This demonstrates that unlike endorphin and morphine, P-gp does not seem to be required for the brain-to-blood transport of the endorphin, Met-enkephalin, or Tyr-MIF-1 across the BBB.

P-Glycoprotein (P-gp) is a transport protein expressed at the capillary endothelial cells that make up the blood-brain barrier (BBB). It can transport a large variety of drugs out of the brain, contributing to what misleadingly seems to be their poor penetration of the BBB (Schinkel, 1999; Terasaki and Hosoya, 1999; Tsuji and Tama, 1999). Knockout mice have been developed for P-gp, which is encoded in the Mdr1a (multidrug resistance 1a) gene (Schinkel et al., 1994). These mice manifest no basic physiological abnormalities (Schinkel et al., 1997), maintain BBB integrity (de Lange et al., 1998), but show increased opiate-induced analgesia (Thompson et al., 2000) and limited transport of morphine (Xie et al., 1999). Recently, the P-gp system was implicated in the transport of i.c.v. administered endorphin from the brain into the blood (King et al., 2001), raising the possibility that other endogenous brain opiates might be similarly transported.

Endorphin has higher affinity and is more selective for the μ-opioid receptor than endorphin (Zadina et al., 1997). A saturable brain-to-blood efflux system has been found for both endorphin-1 and endorphin-2 (Kastin et al., 2001). This shared transport system is not cross-inhibited by Tyr-MIF-1, another opiate-modulating tetrapeptide, which, like the endorphins, we have isolated from brain tissue (Zadina et al., 1989; Hackler et al., 1995). Tyr-MIF-1 has the first saturable transport system across the BBB to be described for a tetrapeptide (Banks and Kastin, 1984); it is extremely stable in cerebrospinal fluid, and its efflux system does not transport any of the peptide fragments contained within the tetrapeptide even though the transporter is shared with Met-enkephalin (Banks et al., 1986, 1990; Kastin et al., 1994). We investigated whether the P-gp system is involved in the transport of the endorphins, Met-enkephalin, and Tyr-MIF-1.

Materials and Methods

Male P-gp knockout mice lacking the Mdr1a gene and their male FVB controls were obtained at about 6 weeks of age from Taconic Farms (German-town, NY). They were housed with free access to food and water with a 12:12-h light/dark schedule. All experiments were reviewed and approved by the Institutional Animal Care and Use Committee. About 25,000 cpm of 125I-endorphin-1 or 125I-endorphin-2 purified by high-performance liquid chromatography, with a mean specific activity about 2100 Ci/mmol, together with 131I-Tyr-MIF-1 (2000 Ci/mmol) were injected into the brain of mice, anesthetized with urethane, at a site 1 mm lateral and 0.2 mm posterior to the bregma with a Hamilton syringe (Banks et al., 1997). The same amount of 125I-Met-enkephalin was used. It has been shown by autoradiography that by this method with these coordinates, material is accurately delivered to the lateral ventricle of the mouse brain (Maness et al., 1998). Binding studies showed that these iodinated endorphins are biologically active.

Mice were decapitated 0, 2, 5, 10, and 20 min after i.c.v. injection, and their brains were removed and counted in a γ-counter. A different mouse was used at each point (n = 4–6/pont). The 0-min value was determined in mice overdosed with anesthesia before injection (Banks and Kastin, 1989). The half-time disappearance was determined from the regression line obtained from the plot of the logarithm of brain radioactivity against time. Groups were compared by analysis of variance, followed by a Duncan’s multiple comparisons test. Regression lines were determined by the least-squares method, and

TABLE 1

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Mouse</th>
<th>t₁/₂ (min)</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endorphin-1</td>
<td>Control</td>
<td>16.34</td>
<td>0.96</td>
</tr>
<tr>
<td>Knockout</td>
<td>14.03</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Endorphin-2</td>
<td>Control</td>
<td>12.43</td>
<td>0.98</td>
</tr>
<tr>
<td>Knockout</td>
<td>12.43</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Met-enkephalin</td>
<td>Control</td>
<td>12.40</td>
<td>0.97</td>
</tr>
<tr>
<td>Knockout</td>
<td>12.43</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Tyr-MIF-1</td>
<td>Control</td>
<td>17.04</td>
<td>0.91</td>
</tr>
<tr>
<td>Knockout</td>
<td>13.00</td>
<td>0.98</td>
<td></td>
</tr>
</tbody>
</table>

1 Abbreviations used are: P-gp, P-glycoprotein; BBB, blood-brain barrier.

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Fig. 1. Efflux of $^{125}\text{I}$-endomorphin-1 from the brains of P-gp knockout mice and their controls.

Fig. 2. Efflux of $^{125}\text{I}$-endomorphin-2 from the brains of P-gp knockout mice and their controls.
Fig. 3. Efflux of $^{125}$I-Met-enkephalin from the brains of P-gp knockout mice and their controls.

Fig. 4. Efflux of $^{131}$I-Tyr-MIF-1 from the brains of P-gp knockout mice and their controls.
For each peptide, the half-time disappearance was not significantly slower for the control group than for the knockout group; if P-gp were involved in the transport of these peptides, the results would have been expected to be significantly different in the opposite direction. The times are shown in Table 1 and the disappearance curves in Figs. 1 to 4. There was no statistically significant difference between any of the pairs. The half-time disappearance of \(^{99m}\)Tc-albumin was 23.72 min (\(r = 0.84\)); unlike the other groups, this slope was not significantly different from zero.

These results show that by contrast with endorphin (King et al., 2001), the endorphins, Met-enkephalin, and Tyr-MIF-1 do not seem to require P-gp for transport out of the brain. This supports our preliminary results showing a lack of significant effect of the P-gp inhibitor cyclosporine injected i.c.v. as a suspension together with the endorphins at a dose of 10 nmol (5 \(\mu g\)/mouse).

P-gp is located in the walls of the lateral ventricle (King et al., 2001) into which our peptides were injected. It is expressed in the choroid plexus but not in P-gp knockout mice (Rao et al., 1999; Warren et al., 2000). The P-gp system has been determined to be “crucial for the analgesic actions of a series of centrally administered opioids” (King et al., 2001), but apparently not for the four opiate-modulating peptides we tested. Based on results in primary cultured choroid epithelial cells (Rao et al., 1999), P-gp in the ventricles may not be optimally oriented to clear substrates injected i.c.v.; thus, caution should be used when interpreting the results, although this did not seem to influence the results (King et al., 2001) with endorphin.

The lack of ability of any fragment of Tyr-MIF-1 to inhibit its exit from the brain shows that the efflux system requires the intact molecule for transport to occur (Banks et al., 1986). The standard technique for demonstrating this involves measurement after i.c.v. injection of the radioactivity remaining in the brain that is not confounded by peripheral degradation in blood or binding to the endothelial cells composing the BBB. The system has been verified by measurement of intact Tyr-MIF-1 and vasopressin in blood after i.c.v. administration (Banks et al., 1987, 1990), and an excellent correlation for several substances exists between the rate of disappearance from the brain and the rate of appearance in blood (Banks and Kastin, 1992). The method accurately quantifies efflux, unlike some other methods that measure efflux only as an index. Moreover, Tyr-MIF-1 incubated in cerebrospinal fluid at 37°C for 24 h remains more than 95% intact (Kastin et al., 1994).

Thus, although the P-gp transport system is active for many drugs as well as endorphin, the transport system shared by endorphin-1 and endorphin-2 and the separate transport system for Tyr-MIF-1 and Met-enkephalin apparently are not affected by the absence of P-gp.

References


