Opioid Modulation of the Fetal Hypothalamic-Pituitary-Adrenal Axis: The Role of Receptor Subtypes and Route of Administration

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ABSTRACT
The role of receptor subtypes in opioid modulation of the hypothalamic-pituitary-adrenal (HPA) axis is well understood in the adult but has not been investigated in the developing fetus. Because the fetal HPA axis plays an important role in the development of several vital organs and in the onset of paruition, an understanding of the role of opioid receptor subtypes on the fetal HPA axis is important in the design of new obstetrical angesics. In these studies, we examined the effects of highly selective mu, delta and kappa opioid agonists on plasma immunoreactive adrenocorticotropic (ir-ACTH) and immunoreactive cortisol (ir-cortisol) in the ovine fetus. Intravenous administration of the mu selective agonist [D-Ala2-N-Me-Phe4,Gly-ol]-enkephalin resulted in a 92% increase in ir-ACTH (P = .005) and ir-cortisol. The delta selective agonist, [d-Pen²,d-Pen⁵]-enkephalin, elicited a much smaller increase (52%) in ir-ACTH (P = .01). In contrast, there was a 7-fold increase in ir-ACTH (P < .001) and a significant increase in ir-cortisol (P = .02) with the kappa selective U50,488H. When the same agonists were administered intracerebroventricularly, there was no change in ir-ACTH or ir-cortisol. These data suggest that the kappa opioid receptor may be more important in the modulation of the fetal HPA axis and that the distribution of these opioid agonists from the lateral ventricle to the hypothalamus and pituitary is very limited.

Opiates and opioid peptides can affect several neuroendocrine systems, and their effects on the HPA axis has been studied extensively in recent years. Acute systemic administration of morphine and other opiates stimulates the release of ACTH from the pituitary and glucocorticoids from the adrenals (for review, see Pechnick, 1993). A similar response is observed after direct administration for morphine into the lateral or third ventricle, which suggests that this stimulatory action of opioids on the HPA axis is centrally mediated. The key central site of action appears to be the hypothalamus. Direct injection of morphine into the hypothalamus results in stimulation of corticosterone release (Lotti et al., 1969) and opiates are capable of stimulating the release of CRF from hypothalami in vitro (Buckingham, 1982; Buckingham and Cooper, 1986).

The hypothalamus contains mu, delta and kappa opioid receptors and they appear to play a differential role in the modulation of the HPA axis. Available evidence suggests that mu and kappa receptors, but not delta receptors, are involved in the opioid-induced stimulation of glucocorticoids and the release of CRF from hypothalami in vitro (Iyengar et al., 1986; Buckingham and Cooper, 1986). Intracerebroventricular administration of the mu selective peptide DAMGO caused an increase in plasma ACTH (Pfeiffer et al., 1985) and corticosterone (Eisenberg, 1993) in rats. The selective kappa agonist, U50,488H, was found to be more potent than morphine in increasing plasma levels of corticosterone even though it is less potent as an analgesic (Hayes and Stewart, 1985; Iyengar et al., 1986). Dynorphin₁₋₁₃ and the kappa selective peptide met-enkephalin-Arg-Phe administered i.c.v. also increased plasma corticosterone (Iyengar et al., 1987; Wood et al., 1987). Administration of the delta selective peptide DPDPE i.c.v., however, did not increase serum levels of corticosterone (Buckingham and Cooper, 1986), and this apparent lack of effect appears to be consistent with relatively few delta receptors in the hypothalamus (Desjardins et al., 1990; Mansour et al., 1993).

ABBREVIATIONS: HPA, hypothalamic-pituitary-adrenal; ACTH, adrenocorticotropic; CRF, corticotropin releasing factor; ir-ACTH, immunoreactive adrenocorticotropic; ir-cortisol, immunoreactive cortisol; U50,488H, trans-(-)-3,4-dichloro-N-methyl-[2-(1-pyridinyl)cyclohexyl]benzeneacetamide; DAMGO, [D-Ala²,N-Me-Phe⁴,Gly-ol]-enkephalin; DPDPE, [D-Pen²,d-Pen⁵]-enkephalin; FK33,824, [D-Ala²,N-Phe⁴, Met(O)³]-enkephalin; BBB, blood-brain barrier; CSF, cerebrospinal fluid; ANOVA, analysis of variance; i.v., intravenous; i.c.v., intracerebroventricular; AVP, arginine vasopressin.
Activation of the HPA axis by opioids and its dependence on the subtype of opioid receptor is present during the early neonatal period. Intravenous U50,488H increased plasma ACTH and corticosterone by postnatal day 2 in the rat, whereas morphine stimulation was not observed until postnatal day 5 (Adamson et al., 1991). On the other hand, DPDPE had no effect on plasma corticosterone in the developing rat (Bero et al., 1987). These observations correlate well with the ontogeny of the opioid receptor subtypes in the rat brain with kappa binding developing first at midgestation, followed by mu binding, and a lack of appreciable delta binding sites until postnatal day 10 (Spain et al., 1985; Pettrillo et al., 1987; Kitchen et al., 1995).

Opioid modulation of the HPA axis may even be apparent before birth. In late-term fetal sheep, i.v. administration of leu-enkephalin resulted in an increase in plasma cortisol levels (Bousquet et al., 1984), and the mu selective enkephalin analog, FK33,824, elevated both plasma ACTH and cortisol levels (Brooks and Challis, 1988). However, the role of receptor subtypes in opioid modulation of the fetal HPA axis has not been examined systematically. Recent studies in our laboratory suggest that kappa opioid agonists have a profound stimulatory effect on plasma ACTH and cortisol levels in the late-term fetal sheep (Taylor et al., 1996). Cortisol plays an important role in the onset of parturition as well as the maturation of several fetal organ systems, including the lung and kidney, that are essential for extraterrestrial survival (Magyar et al., 1980). An understanding of the role of opioid receptor subtypes on the fetal HPA axis is important in the design of opioid drugs for obstetrical uses. In this study, we compared the effects of i.v. DAMGO, DPDPE and U50,488H on fetal plasma ACTH and cortisol levels in the late-term ovine fetus. In addition, because the distribution of the enkephalin peptide analogs across the BBB may be rather limited, we have also examined the effects of these agonists after i.c.v. administration.

Materials and Methods

Surgical procedure. Fetal sheep of mixed Western breed (gestational age ~115 days, 0.8 of term) were surgically instrumented with chronic indwelling polyvinyl catheters as described in detail by Szeto et al. (1990). For drug infusion, a catheter was inserted into the fetal inferior vena cava via the femoral vein, and another catheter was placed in the lateral cerebral ventricle. For blood sampling, a catheter was placed into the femoral artery and advanced to the distal aorta. Guidelines approved by the Institution for the Care and Use of Animals at Cornell University Medical College were followed for all surgical procedures and experimental protocols.

Experimental protocol. Experiments were performed a minimum of 5 days after surgery to ensure complete recovery from surgical stress. On the day of the study, the ewe was placed in a mobile cart in a quiet room at approximately 8:00 A.M. with free access to food and water through the duration of the study. The ewe was allowed a 2-h acclimation period during which a control sample of fetal blood (2 ml) was collected for determination of basal arterial blood gases and pH (Radiometer ABL30, Cleveland, OH) as well as basal ir-ACTH and ir-cortisol levels.

DAMGO (Multiple Peptide Systems, San Diego, CA; 0.15 and 0.3 mg/kg/h) and DPDPE (Multiple Peptide Systems, San Diego, CA; 0.3 mg/kg/h) were administered i.v. to the fetus for 1 h, and fetal blood samples were collected before, during and at the end of the infusion, and 1 h and 3 h after drug infusion. U50,488H (Research Biochemical International, Natick, MA; 0.5 and 1.0 mg/kg) was administered to the fetus i.v. for 1 min, and blood samples collected before, and at 15, 30 and 60 min after drug administration. The doses of DAMGO and DPDPE are about 10-fold higher than the i.c.v. doses previously used in the study of their cardiorespiratory and EEG effects on the ovine fetus (Szeto et al., 1990, 1994, 1995). The doses of U50,488H were based on a previous report (Taylor et al., 1996).

For the i.c.v. studies, DAMGO (0.003 and 0.03 mg/kg/h), DPDPE (0.01 and 0.03 mg/kg/h) and U50,488H (0.06 mg/kg/h) were administered at a constant rate for 1 h and blood samples were collected before and at the end of infusion, and 1 h and 3 h after the termination of drug infusion. The doses for DAMGO and DPDPE were based on previous studies (Szeto et al., 1990, 1994, 1995), and the dose for U50,488H was approximately 10-fold lower than the i.v. dose reported previously (Taylor et al., 1996).

An incomplete randomized cross-over design was used in the administration of the various drug treatments with each fetus receiving no more than three different treatments with a minimum of 2 days between studies. Gestational age on the day of the study ranged from 122 to 142 days.

Radioimmunoassay. Blood samples were centrifuged at 1500 rpm for 10 min at 4°C and subsequently frozen at −70°C until assayed. [125I]ACTH and [125I]cortisol radioimmunoassay kits (INCSTAR, Stillwater, MN) were used to measure fetal plasma ir-ACTH and ir-cortisol concentrations. These assays have been standardized in our laboratory by use of ovine fetal plasma (Taylor et al., 1996).

Statistical analysis. All data are reported as mean ± S.E.M. A single-factor ANOVA with repeated measures (factor = time) was used to determine the effect of drug treatment on the change in fetal plasma ir-ACTH and ir-cortisol levels. Friedmans repeated measures ANOVA on ranks was used if the normality test failed. Dunnett’s post hoc comparison was used to determine the time at which a significant change from control values was attained. Statistical significance was set at P < .05.

Results

Basal levels of ir-ACTH and ir-cortisol. Basal levels of ir-ACTH and ir-cortisol were within normal range and are summarized in table 1. No significant differences were observed among the basal ir-ACTH levels. However, large variations were observed in basal ir-cortisol levels, and this is most likely caused by the progressive increase in responsiveness of the fetal adrenal to ACTH in the last 10 to 15 days of gestation (Magyar et al., 1980). Neither i.v. nor i.c.v. administration of saline had any effect on plasma ir-ACTH or ir-cortisol.

effects of i.v. DAMGO on plasma ir-ACTH and ir-cortisol. Intravenous administration of DAMGO resulted in a dose-related increase in plasma ir-ACTH levels (fig. 1). The higher dose of 0.3 mg/kg/h resulted in a significant increase in ir-ACTH (F = 5.55; P = .005), with a peak increase of 91.5 ± 19.9% during the infusion, followed by a rapid decline to control levels 1 h later. Plasma ir-cortisol increased from 4.2 ± 1.0 ng/ml to 8.3 ± 2.8 ng/ml but did not achieve statistical significance.

effects of i.v. DPDPE on plasma ir-ACTH and ir-cortisol. Intravenous DPDPE infusion (0.3 mg/kg/h) also resulted in a significant increase in ir-ACTH (F = 4.92; P = .01), with a peak increase of 51.5 ± 14.6% at 15 min (fig. 2). The increase in plasma ir-cortisol did not reach statistical significance.

Effects of i.v. U50,488H on plasma ir-ACTH and ir-cortisol. Intravenous U50,488H produced a dose-dependent increase in plasma ir-ACTH which lasted for at least 3 h after bolus administration (fig. 3). These data were reported in an
earlier publication (Taylor et al., 1996). The lower dose (0.5 mg/kg) produced a 3- to 4-fold increase in ir-ACTH at 30 min ($F = 5.21; P = .04$), but the increase in ir-cortisol did not reach statistical significance ($F = 3.94; P = .07$). The higher dose (1.0 mg/kg) produced a 7-fold increase in ir-ACTH at 1 h, which was highly significant ($F = 20.8; P < .001$), and was associated with a significant increase in ir-cortisol ($\chi^2 = 9.96; P = .01$). Plasma ir-ACTH declined from there but was still slightly above control levels at 3 h (data not shown).

**TABLE 1**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dose</th>
<th>n</th>
<th>Gestational age</th>
<th>ir-ACTH (pg/ml)</th>
<th>ir-cortisol (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.v. saline</td>
<td>0.15 mg/kg/h</td>
<td>4</td>
<td>128 ± 2</td>
<td>32.3 ± 3.1</td>
<td>7.7 ± 1.3</td>
</tr>
<tr>
<td>i.v. DAMGO</td>
<td>0.3 mg/kg/h</td>
<td>5</td>
<td>125 ± 2</td>
<td>22.3 ± 1.2</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>i.v. DAMGO</td>
<td>0.3 mg/kg/h</td>
<td>4</td>
<td>127 ± 1</td>
<td>25.4 ± 3.2</td>
<td>4.2 ± 1.0</td>
</tr>
<tr>
<td>i.v. DAMGO</td>
<td>0.5 mg/kg/h</td>
<td>3</td>
<td>132 ± 3</td>
<td>30.3 ± 3.0</td>
<td>6.2 ± 2.2</td>
</tr>
<tr>
<td>i.v. DAMGO</td>
<td>1.0 mg/kg/h</td>
<td>5</td>
<td>135 ± 5</td>
<td>23.0 ± 1.4</td>
<td>30.2 ± 6.9</td>
</tr>
<tr>
<td>i.v. DAMGO</td>
<td>0.03 mg/kg/h</td>
<td>6</td>
<td>135 ± 3</td>
<td>44.3 ± 6.2</td>
<td>15.9 ± 4.4</td>
</tr>
<tr>
<td>i.v. DAMGO</td>
<td>0.03 mg/kg/h</td>
<td>7</td>
<td>131 ± 3</td>
<td>28.9 ± 3.0</td>
<td>14.7 ± 4.7</td>
</tr>
<tr>
<td>i.v. DAMGO</td>
<td>0.003 mg/kg/h</td>
<td>6</td>
<td>129 ± 2</td>
<td>n.d.*</td>
<td>6.2 ± 0.6</td>
</tr>
<tr>
<td>i.v. DAMGO</td>
<td>0.01 mg/kg/h</td>
<td>5</td>
<td>132 ± 2</td>
<td>31.6 ± 2.7</td>
<td>17.7 ± 2.4</td>
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<td>0.03 mg/kg/h</td>
<td>4</td>
<td>134 ± 2</td>
<td>36.1 ± 2.6</td>
<td>33.0 ± 12.3</td>
</tr>
<tr>
<td>i.v. DAMGO</td>
<td>0.03 mg/kg/h</td>
<td>6</td>
<td>135 ± 3</td>
<td>31.7 ± 6.6</td>
<td>15.0 ± 10.3</td>
</tr>
<tr>
<td>i.v. U50,488H</td>
<td>0.06 mg/kg/h</td>
<td>6</td>
<td>129 ± 3</td>
<td>44.5 ± 7.2</td>
<td>17.3 ± 5.4</td>
</tr>
</tbody>
</table>

* n.d., not determined.

**Fig. 1.** Effects of i.v. DAMGO (●, 0.15 mg/kg/h; ■, 0.3 mg/kg/h) on fetal plasma (A) ir-ACTH and (B) ir-cortisol. DAMGO was administered as a 1-h infusion beginning at time 0 h. $n = 5$. *$P < .05$ as compared with predrug control levels.

**Fig. 2.** Effects of i.v. DPDPE (●, 0.3 mg/kg/h) on fetal plasma (A) ir-ACTH and (B) ir-cortisol. DPDPE was administered as a 1-h infusion beginning at time 0 h. $n = 4$. *$P < .05$ as compared with predrug control levels.

**Effects of i.v. DAMGO on plasma ir-ACTH and ir-cortisol.** Intracerebroventricular infusion of DAMGO had no effect on plasma ir-ACTH or ir-cortisol during the infusion, even with a dose of 0.03 mg/kg/h (data not shown). There was a small increase in ir-ACTH in four of the seven animals 3 h after termination of the i.c.v. infusion, which was associated with an increase in ir-cortisol. However, these changes were not statistically significant because of the large variation in response.
Effects of i.c.v. DPDPE on plasma ir-ACTH and ir-cortisol. Neither the 0.01 mg/kg/h nor 0.03 mg/kg/h dose of i.c.v. DPDPE resulted in a significant change in ir-ACTH or ir-cortisol (data not shown).

Effects of i.c.v. U50,488H on plasma ir-ACTH and ir-cortisol. Intracerebroventricular infusion of U50,488H, even at a dose of 0.06 mg/kg/h, had no effect on either ir-ACTH or ir-cortisol (data not shown).

Comparison of i.v. and i.c.v. administration of DAMGO, DPDPE and U50488. The changes in plasma ir-ACTH levels after i.v. and i.c.v. administration of DAMGO, DPDPE and U50488 at the time of peak effect are summarized in fig. 4. Significant increases in ir-ACTH were only observed after i.v. administration of these opioid agonists.

Discussion

The actions of exogenously administered opioids on plasma ACTH and cortisol levels in the adult sheep are inconclusive. Intravenous administration of met-enkephalin and the mu selective FK33,824 both caused an increase in plasma concentration of ACTH (Wang et al., 1986). In other studies, i.v. injection of leu-enkephalin apparently had no effect on plasma cortisol in adult sheep (Bousquet et al., 1984), and i.v. administration of met-enkephalin or FK33–824 did not alter ACTH or cortisol secretion (Brooks and Challis, 1989). In contrast, Redekopp et al. (1985) reported that i.v. FK33–824 inhibited pituitary-adrenal function. Furthermore, Parrott and Goode (1993) found no effect on plasma cortisol when morphine, dynorphin or [D-Ala²,D-Leu⁵] enkephalin (DADLE) were injected i.c.v., whereas Wang et al. (1988) reported that i.c.v. infusion of met-enkephalin and FK33–824 actually decreases basal levels of ACTH. These discrepancies may be caused partly by the use of different routes of administration, dose and the endocrine state of the animal. The relative lack of selectivity of the opioid agonists used also precludes any conclusions from being made regarding the differential role of mu, delta and kappa receptors in modulation of the HPA axis.

In the present study, highly selective agonists were used to ascertain the role of mu, delta and kappa receptors in modulation of the ovine fetal HPA axis. The ontogeny of the ovine fetal HPA axis has been studied extensively, and it is known that fetal pituitary corticotrophs are responsive to CRF by 97 days of gestation, although the fetal adrenal is not responsive to ACTH until 125 days (Challis and Brooks, 1989). The present studies were therefore carried out in fetal lambs greater than 125 days’ gestation. Our results show that the primary effect of these opioid agonists appears to be on the stimulation of ACTH release. Furthermore, the magnitude of response was dependent not only on the specific opioid agonist but also on the route of administration. This is clearly demonstrated in figure 4 which summarizes the change in ir-ACTH after i.v. and i.c.v. administration of DAMGO, DPDPE and U50,488H.

The administration of all three opioid agonists by the i.v. route resulted in a release of ACTH, although the magnitude of ir-ACTH release differed among the three agonists and was most pronounced with U50,488H. DAMGO resulted in a significant increase in ir-ACTH that was approximately 92% of control values, whereas the same dose of DPDPE only increased ir-ACTH by 52%. The magnitude of ir-ACTH increase with DAMGO is comparable with that observed when 5.0 mg/h of morphine was administered i.v. to the ovine fetus (unpublished data). This dose of morphine is considered a high dose in the fetal lamb, because it profoundly affects fetal heart rate (Zhu and Szeto, 1989), fetal breathing (Szeto et al., 1988), fetal electrocortical activity (Szeto, 1991) and glucose regulation (Szeto et al., 1995). Intravenous U50,488H, on the
other hand, elicited an extremely robust and long-acting stimulation of ir-ACTH release, which was approximately 7-fold compared with control levels. At this dose, U50,488H significantly increased fetal blood pressure and heart rate and depressed fetal breathing, and these effects also lasted for 2 to 3 h (Szeto et al., 1996). The concurrent increase in plasma ir-cortisol suggests that at least some of the ACTH released was of the bioactive form. The variation in cortisol response most likely reflects the normal variation in responsiveness of the fetal adrenal to ACTH, which is a function of gestational age (Challis and Brooks, 1989).

The difference in fetal ACTH responses to the three opioid agonists suggests that mu, delta and kappa agonists may have different abilities in modulating the fetal HPA axis or it could represent ontogenetic differences in the development of the three subtypes of opioid receptors in the ovine fetal brain. Both adult and neonatal studies have shown that kappa agonists are more potent than mu agonists in stimulating the HPA axis, and that delta agonists have no effect (Pechnick, 1993; Adamson et al., 1991; Bero et al., 1987). The differential ability of mu, delta and kappa agonists in stimulating ACTH release in adult and neonatal rats is consistent with the distribution of these three receptor subtypes in the hypothalamus as well as the ontogenetic pattern of these three receptor subtypes in the neonatal rat. In the adult rat, there is a high density of kappa binding sites in the supraoptic and paraventricular nuclei of the hypothalamus, with a comparatively lower density of mu sites and virtually no delta sites (Mansour et al., 1995). Binding studies in the neonatal rat revealed that adult levels are reached between 7 and 14 days after birth for the kappa site, between 14 and 21 days for the mu site, whereas only 50% of adult levels are reached by 21 days for the delta sites (Spain et al., 1985; Petrillo et al., 1987; Kitchen et al., 1995). Opioid binding sites have been demonstrated in the ovine fetus from gestational day 110, reaching adult levels by 125 days gestation, and they are present in the hypothalamus but not the pituitary (Pfeiffer et al., 1982; Qi et al., 1990). Ontological studies, however, have not been carried out with highly selective mu, delta and kappa ligands in the ovine fetus.

The more limited ACTH response to DAMGO and DPDPE may also reflect their more restricted distribution across the BBB rather than a limited role for mu and delta receptors in the modulation of the HPA axis. The transport of opioid peptides into the brain after i.v. administration has been a matter of some controversy (Kastin et al., 1976; Conford et al., 1978). Several studies have now examined the ability of enkephalin analogs to cross the BBB (Banks and Kastin, 1985; Weber et al., 1992). When [3H]DPDPE was administered i.v., approximately 0.95 to 0.1% of total radioactivity was found in brain tissues at 10 to 30 min (Weber et al., 1992). This surprisingly was not very different from the amount of morphine previously reported to be in the brain (0.1%) after i.v. administration (Oldendorf et al., 1972). The blood-brain ratio for met-enkephalin and leu-enkephalin after intracarotid injection was calculated to be approximately 48 to 70 (Banks and Kastin, 1985). It is now quite clear that small amounts of opioid pentapeptides do cross the BBB (Banks and Kastin, 1990), and i.v. administration of DPDPE can elicit analgesia in mice (Weber et al., 1992). To optimize the distribution of these peptides into the central nervous system, and to avoid peptide degradation, we chose to maintain plasma peptide levels with a 1-h infusion rather than with the use of a bolus injection. In comparison with U50,488H, however, the penetration of these two peptides across the BBB is probably still quite restricted. It should be noted, however, that the median eminence and pituitary lack a BBB and systemically administered peptides may penetrate these parts with ease.

To improve their entry into the central nervous system, these opioid peptide analogs are generally administered into the lateral cerebroventricle. We have therefore compared the effects of these opioid agonists after i.c.v. administration. To maintain steady state levels in the ventricular system, all drugs were administered as a constant rate infusion over 1 h. When given i.c.v., we were surprised by their lack of efficacy even though the i.c.v. doses were only approximately 10-fold lower than the corresponding i.v. doses. Assuming the extent of distribution for DPDPE as reported by Weber et al. (1992), these doses should have resulted in brain levels that are far in excess of those that could be accomplished by the i.v. route. One interpretation of our results could be a very slow distribution of these peptides from CSF to the hypothalamus, which is supported by the increase in ir-ACTH observed in some animals 3 h after the termination of the i.c.v. infusion. Although i.c.v. injection of [3H]DAMGO into the lateral ventricle of the rat led to widespread distribution of radiolabel into the third ventricle, the label was found to only diffuse 1 to 2 mm from the third ventricle into adjoining brain areas 30 min after the injection (Aloyo et al., 1993). In addition, a carrier-mediated transport system, termed peptide transport system 1, has been described for small, N-tyrosinated peptides, including met-enkephalin, which may provide rapid egress of these peptides from the ventricular system (Banks and Kastin, 1984). Thus the diffusion of DAMGO and DPDPE across the CSF-brain barrier may be so slow that most of the peptide could have been removed by the choroid plexus before any significant amount diffuses across into hypothalamic tissue. Interestingly, the higher dose of DAMGO has been shown to significantly increase fetal heart rate (Szeto et al., 1990), depress fetal breathing (Szeto et al., 1995) and increase fetal plasma glucose and lactate levels (Szeto et al., 1995). Similarly, i.v. administration of DPDPE has been known to stimulate fetal breathing (Cheng et al., 1992) and electrocortical activity (Szeto et al., 1994), although it had no effect on fetal heart rate (Szeto et al., 1990) or glucose regulation (Szeto et al., 1995). Thus it appears that the distribution of these opioid peptides into different brain regions from the lateral ventricle is not uniform, making the interpretation of i.c.v. data very difficult.

Route of administration also played an important role in the action of U50,488H on the HPA axis. U50,488H resulted in a highly robust increase in ir-ACTH and ir-cortisol after i.v. administration but was entirely unable to elicit a response when administered via the i.c.v. route. In a recent study, we found that the stimulatory action of U50,488H on the pituitary-adrenal axis was mediated, at least in part, by the release of CRF and AVP from the hypothalamus (Taylor et al., 1996). It was therefore rather surprising that U50,488H was completely ineffective in modulating plasma ir-ACTH when administered i.c.v. A possible interpretation is that if a compound is highly lipid soluble, very little will be found in brain tissues because the material will rapidly pass into the blood stream via the first few capillaries beneath the
ependyma or that diffusion into the brain may be more rapid than bulk flow of CSF so that little drug reaches the third ventricle. U50,488H, like other organic bases, may also be rapidly taken up by the choroid plexus and removed from CSF into blood. A similar discrepancy between systemic and i.c.v. administration has been described for U69,593 in which s.c. administration resulted in a decrease in plasma oxytocin and AVP, but i.c.v. administration was without effect (Van de Heijning and Van Wimersma Greidanus, 1994). Although this was interpreted by the authors as indicative of a peripheral site of action, there is evidence that kappa agonists suppress AVP at the level of the hypothalamus (Rossi and Brooks, 1996). Finally, although the metabolism of U50,488H in vivo has not been investigated, the slow rise to reach peak response after i.v. administration suggests that its actions on the HPA axis may be mediated via an active metabolite and the lack of effect of i.c.v. administration may reflect the inability of the metabolite to be formed in the brain or CSF.

With respect to the HPA axis, differential actions of the same opioid agonists and antagonists depending on the route of administration have been reported. In the adult rat, a rise in corticosterone was observed after i.v. administration of [d-Ala²,Me⁵] enkephalinamide (Tortella et al., 1979), whereas administration directly into the third ventricle had no effect on either ACTH or corticosterone levels (Pinsky et al., 1978). In adult sheep, i.c.v. naloxone administration was ineffective in blocking the effects of exogenously administered opioids, whereas i.v. naloxone abolished the response (Parrott and Goode, 1993). In this same study, i.c.v. administration of morphine, dynorphin and DADLE had no effect on cortisol secretion, although clearly i.v. administration of the same or similar opioids can provoke cortisol release (Redekopp et al., 1985; Parrott and Thornton, 1989; Parrott and Goode, 1992). In addition, studies have demonstrated a stimulation of the fetal sheep HPA axis by i.v. FK33–824 (Wang et al., 1986) and an inhibition after i.c.v. administration of the same compound (Wang et al., 1988). Taken together with the inherent variables of i.c.v. administration such as the placement of the catheter into the lateral ventricle, the volume of the ventricle and the circulation of the CSF, these studies implicate the i.v. route of administration of opioid peptides as a more reliable, and perhaps, more efficacious mode of delivery.

With the evidence for lower density of mu and delta binding within the hypothalamus and the later development of these receptors with respect to kappa receptors, it is likely that the differential response to the three selective opioid agonists observed in our study represents a more prominent role of the kappa opioid receptor in modulation of the fetal HPA axis. Our results are consistent with other reports which have shown that kappa opioid agonists stimulate the HPA axis to a greater extent than mu or delta agonists. The 7-fold stimulated increase in ir- ACTH after U50,488H administration in our study is comparable with what has been seen in the adult rat after the administration of another selective kappa agonist, MR-2034, contrasting with morphine-induced stimulation of less than 2-fold (Pfeiffer et al., 1985). Of greater relevance is the study by Adamson and co-workers who showed, in 10-day-old rat pups, an 8-fold increase in plasma immunoreactive corticosterone after U50,488H as compared with a 3-fold stimulation by morphine (Adamson et al., 1991). It is likely, therefore, that the kappa opioid system plays a more prominent role in modulation of the ovine fetal HPA axis than the mu or delta systems.

Finally, these results show that route of administration can significantly influence the outcome of studies comparing selective opioid agonists in intact animals, and they suggest that caution must be exercised in the interpretation of any i.c.v. data.

References


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