Insulin-Like Growth Factor-II Uptake Into Choroid Plexus and Brain of Young and Old Sheep

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Insulin-like growth factor II (IGF-II) is a major growth factor in brain and is involved in neuroprotection in later life. However, synthesis and delivery of IGF-II to brain by the choroid plexus (CP) in later life is not well understood. This study investigated these issues in old sheep (7–10 years) in comparison to young adult sheep (1–2 years). IGF-II messenger RNA expression at the CP did not change with age although cerebrospinal fluid (CSF) levels fell. $^{125}$I-IGF-II uptake in the CP was saturated from either side of the CP, whereas age-related decrease of the uptake was seen at the CSF side but not at the blood side of the CP. The insulin-like growth factor binding protein-2 (IGFBP-2) at 0.01 or 0.1 μg/mL tended to enhance IGF-II uptake at the young CP but not the old CP or other brain tissues, whereas bovine serum albumin generally inhibited the uptake. These age-related changes suggest that the normal autocrine/paracrine role of IGF-II at the CP is attenuated with age.

Key Words: Insulin-like growth factor II—Choroid plexus—Aging—Brain—Insulin-like growth factor binding protein-2.

The insulin-like growth factor (IGF) system consists of two IGFs (-I and -II), six IGF binding proteins (IGFBP 1–6) and two IGF receptors (IGFR-I and IGFR-II/cation-independent mannose 6-phosphate receptor [M6P-R]) (1), and has important roles in human development (2), homeostasis (1,3), and aging (4,5). IGFs themselves are synthesized largely in the liver, from where they are released directly into the circulatory system, displaying endocrine activity (1). However, smaller quantities of IGFs can be synthesized locally within many tissue types, where they display paracrine or autocrine regulatory properties (6). IGF-II gene expression stops or is very low in all tissues of the adult rat and sheep with the exception of the brain (6–8), indicating the importance of IGF-II in the brain after development (9). IGF-II transcription in the brain is mainly localized at the choroid plexus (CP) and leptomeninges (10). The ovine CP epithelium synthesizes both IGF-II and its preferential binding protein, IGFBP-2, and secretes them both into cerebrospinal fluid (CSF) (11), which is thought to account for their high levels in CSF compared to IGF-I and other IGFBPs. CP-derived IGF-II is an autocrine regulator of epithelial growth in the rat (12), and together with IGF-I may exert important endocrine-like effects in the adult rat and human brain (13,14). IGF-II has also been implicated as a regulator of cellular responses in the traumatized rat brain (15). In the acute phase of rat brain injury, IGF-II secretion into CSF by the CP is increased (15), giving it an endocrine-like role. During the chronic phase, IGF-II expression is increased focally around the wound, helping to restore tissue homeostasis (15), when IGF-II reverts back to its autocrine/paracrine role. IGF-II has also been implicated in protection against Alzheimer’s disease (AD)-related neuronal injury in mouse and human models (16,17).

During normal aging and later life, circulating growth hormone and IGF-I decline by about 14% per decade (18,19); this decline is related to age-related learning and memory deficiencies in humans, rats, and mice (20–22) and increased incidence of age-dependant AD (23,24). Because circulating IGF-I upregulates rat brain IGF-II messenger RNA (mRNA) (25), the protective effects of IGF-I may be mediated in part by regulation of IGF-II. In contrast to IGF-I, relatively little is known about age-related changes to IGF-II in brain, despite IGF-II being present at significant levels compared to IGF-I (26). In this study, we compared IGF-II concentrations in CSF and plasma, IGF-II gene expression, transport at CP and uptake into isolated brain tissues in old and young adult sheep, and the influence of IGFBP-2.

METHODS

All procedures were within the guidelines of the Animals (Scientific procedures) Act, U.K., 1986. Clun Forest strain sheep between 1 and 10 years old were used, and divided into two groups: aged 1–2 years (young adult sheep, 25–35 kg) and 7–10 years (old sheep, ~60 kg). The average life expectancy and maximum life span of domestic sheep are 7.1 and 12 years, respectively (27), and old sheep ages ranged from 7 to 10 years (approximately 60%–80% maximum life span with human equivalents of 70–100 years). Young sheep reach reproductive maturity relatively faster than humans, mating at 7–9 months old (gestation is 5 months) (28); therefore, 15- to 27-month-old sheep were chosen as young adults. The sheep were anesthetized with intravenous thiopentone sodium (20 mg/kg) and received an injection of...
apolis, IN) was used for all reactions according to the manufacturer’s instruction. The MgCl₂ concentration was optimized for each primer pair to obtain a single-peak melting curve. After an initial 10-minute preincubation at 95°C, the mixture was subjected to 42 cycles of a three-step PCR, comprising 15 seconds of denaturation at 95°C, a 4-second annealing phase at 58°C, and a 6-second elongation phase at 72°C. Delta cycle threshold (ΔCt) values were calculated for IGF-II in each sample after normalization with the housekeeping gene, GAPDH.

**CP Uptake of ¹²⁵I-IGF-II from Blood Face Using In Situ Perfusion**

The in situ perfused ovine CP method has been previously described in full (29). In brief, after removal of brain, the circle of Willis supplying the choroidal arteries to each lateral ventricle CP were cannulated and perfusion commenced with Ringer’s solution (in mM: NaCl 123, KCl 4.8, Na₂HPO₄ 1.22, CaCl₂ 2.4, MgSO₄ 1.22, NaHCO₃ 25, glucose 5), containing 4% dextran at 37°C and gassed with 95% O₂ and 5% CO₂ for 3–5 hours. At the beginning and end of each experiment, the rate of CSF secretion by the preparation was calculated from the arterial–venous difference in Evans Blue albumin concentration (29). The Ringer outflow was collected from the cannulated great vein of Galen, into which the veins from each CP flow. The cerebral hemispheres were opened to gain access to the CSF side of the CPs, and they were kept moist by constantly flowing artificial CSF (aCSF) (in mM: NaCl 148, KCl 2.5, CaCl₂ 2.5, MgCl₂ 1.8, NaHCO₃ 26) at 37°C and were gassed with 95% O₂ and 5% CO₂. A 100-µL bolus containing 18.5 kBq (2.33 nM) ¹²⁵I-IGF-II (Amersham Biosciences UK Limited) and 18.5 kBq (92.5 nM) extracellular marker ¹⁴C-mannitol (PerkinElmer, Waltham, MA) was added to the perfusion inflow via a series of taps without increasing pressure. Single-drop samples of venous effluent were then collected over approximately 1 minute. The radioactivity of the venous effluent and bolus was counted by liquid scintillation (Rackbeta Spectral 1219 counter; LKB Wallac, Turku, Finland) after the addition of 3.5 mL of liquid scintillation fluid (Ultima Gold; PerkinElmer). The uptake of ¹²⁵I-IGF-II, relative to ¹⁴C-mannitol, for each drop was calculated (29,30). The maximal cellular uptake (Uₘₐₓ) was determined from the samples of venous effluent, in which the ¹⁴C and ¹²⁵I activities had reached peak levels after bolus injection in the first minute (30). The total average uptake over this time and an additional 3 minutes (Uₙₐ₃ (30) and any backflux (Uₘₐₓ – Uₙₐ₃) were also calculated. For kinetic analysis of IGF-II uptake, different concentrations of unlabeled recombinant human IGF-II (VWR International Ltd, Lutterworth, U.K.) at 50, 100, 150, 200, or 300 nM were added to the perfusion fluid. Because the injectate bolus undergoes some mixing with the Ringer before reaching the plexuses, the unlabeled IGF-II concentration reaching the CPs was estimated by calculating a dilution factor (30), which was 5.20 ± 2.10 (n = 3) and 5.32 ± 0.3 (n = 4) in the young and old CP perfusions, respectively. The flux was then calculated (29,30). Plots of concentration versus flux were fitted by nonlinear regression analysis (EnzFitter program; Biosoft, Cambridge, U.K.) to calculate the

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**Table 1. Primers Used for Real-Time PCR**

<table>
<thead>
<tr>
<th>Gene (bp)</th>
<th>Primer Designated Name</th>
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<tbody>
<tr>
<td>IGF-II (115)</td>
<td>5'-CCACCATCTACTGCGCGCT-3' (forward) IGFIS1</td>
</tr>
<tr>
<td></td>
<td>5'-TGAAGGATGTTGGGTCGCGG-3' (reverse) IGFIIA</td>
</tr>
<tr>
<td>GAPDH (142)</td>
<td>5'-AAGATGTCAGCAATGTCCT-3' (forward) GPS1</td>
</tr>
<tr>
<td></td>
<td>5'-CGGAGGACCATCCACTCTT-3' (reverse) GPA7</td>
</tr>
</tbody>
</table>

Note: PCR = polymerase chain reaction; IGF-II = insulin-like growth factor 2; GAPDH = glyceraldehyde 3-phosphate dehydrogenase.
half-saturation constant \( K_m \) (nM), the maximal flux \( V_{\text{max}} \)
(pmol/min/g), and the diffusion coefficient \( K_d \) (mL/min/g).

**Isolated Tissue Incubations**

In another four pairs of young and old sheep, samples of lateral ventricle CP, ventricular ependymal layer (EP), frontal cortex (CX), and hippocampus (HP) were quickly taken after the brain was released from the skull, and were divided into fine flat pieces with similar size weighing between 10 and 20 mg. These tissues were immediately incubated in Eppendorf tubes containing 0.5 mL of aCSF plus 5 mM NaClO4 to block any potential free iodine transport to brain (31), with 2.31 kBq \(^{125}\text{I}-\text{IGF-II} \) (0.0467 nM) and 4.63 kBq \(^{14}\text{C-mannitol} \). In other groups, the aCSF also contained unlabeled IGF-II (at 2.2 \( \mu \text{g/mL} \), IGFBP-2 (at 0.01, 0.1, 0.5, or 1.0 \( \mu \text{g/mL} \)), or bovine serum albumin (BSA; at 0.01, 1, 250, or 400 \( \mu \text{g/mL} \)) (Sigma, St. Louis, MO). The aCSF was prewarmed to 37°C in a water bath, and gassed continuously with 95% \( \text{O}_2 \) and 5% \( \text{CO}_2 \) via a 25-gauge needle through the Eppendorf cap. After incubation for 30 minutes, the tissues were removed and rinsed rapidly (1 second) in clean aCSF, wiped over a glass slide to remove adhering fluid, placed on a foil boat, and weighed on a Cahn Microbalance (Thermo Scientific, Waltham, MA) at 15-second intervals. The tissues were dissolved in 200 \( \mu \text{L} \) of tissue solubilizer (Solusol; National Diagnostics, Atlanta, GA), and the radioactivity was counted by liquid scintillation after the addition of 3.5 mL of liquid scintillation fluid. The integrity of radiolabel was assessed by trichloroacetic acid precipitation on aCSF \(^{125}\text{I}-\text{IGF-II} \) samples before and after incubation, with >95% of radioactivity present in the precipitate following incubation. Net IGF-II uptake was determined from the volume of distribution, \( V_d \) (\( \mu \text{L/mg} \)) for \(^{125}\text{I}-\text{IGF-II} \) after correction for extracellular \(^{14}\text{C-mannitol} \),

\[
V_d = \left( \frac{^{125}\text{I dpm} \cdot \text{mg}^{-1} \text{ tissue}}{^{125}\text{I dpm} \cdot \mu \text{L}^{-1} \text{ aCSF}} \right) - \left( \frac{^{14}\text{C dpm} \cdot \text{mg}^{-1} \text{ tissue}}{^{14}\text{C dpm} \cdot \mu \text{L}^{-1} \text{ aCSF}} \right).
\]

**Data Analysis**

All values were expressed as mean ± standard error of the mean (SEM). Unpaired \( t \) test, or analysis of variance (ANOVA) with post hoc test (Bonferroni) was applied as appropriate to compare means using SPSS 11 (Chicago, IL). Values of \( p < .05 \) were considered statistically significant.

**Results**

**IGF-II Concentration in CSF and Plasma**

CSF levels of IGF-II were approximately one quarter of plasma levels (Figure 1) in young sheep. With age, IGF-II decreased significantly in old CSF but not in plasma.

**IGF-II Gene Expression at CP**

Real-time PCR with primers IGFIIAS1 and IGFIIA showed that IGF-II gene expression in the CP was similar in young and old sheep (Figure 2A). The \( \Delta \text{Ct} \) values for IGF-II gene expression were not different between the two groups (Figure 2B).

![IGF-II UPTAKE INTO CP](https://academic.oup.com/biomedgerontology/article-abstract/63/2/141/632023)

**Kinetics of \(^{125}\text{I}-\text{IGF-II} \) Transport at the Blood Face of CP**

Using the perfused CP preparation, the calculated CSF secretion rate averaged 97.3 ± 12.6 \( \mu \text{L/min/g} \) (\( n = 5 \)), which is consistent with a previous study (30) and indicates viability of the preparation. In the absence of unlabeled IGF-II, \(^{125}\text{I}-\text{IGF-II} \) \( U_{\text{max}} \) in the first minute and \( U_{\text{av}} \) over 4 minutes were not different between the two groups (Table 2). The backflux over 4 minutes was small, suggesting internalization of IGF-II, rather than simple binding to plasma membranes. In the presence of unlabeled IGF-II, \( U_{\text{max}} \) decreased as the concentration of unlabeled IGF-II increased, indicating receptor-mediated IGF-II transport at the basolateral (blood) face of the ovine CP. Figure 3 shows a plot of total concentration of IGF-II (both labeled and unlabeled) versus flux, which could be described by Michaelis–Menten-type kinetics. Neither \( V_{\text{max}} \) nor \( K_m \) differed between the young and old sheep. \( K_p \) in both groups was not different from zero (Table 2).

**\(^{125}\text{I}-\text{IGF-II} \) Uptake into Isolated Brain and CP**

When isolated tissues were incubated in aCSF, the volume of distribution (\( V_d \)) of \(^{125}\text{I}-\text{IGF-II} \) was positive in all tissues (Figure 4). Two-way ANOVA indicated significant differences with age \( (F = 4.592, \ p = .039) \) and by region \( (F = 7.398, \ p = .001) \). These differences could be accounted for by the CP uptake, which was twice that of other brain regions and was greater in the young sheep than in the old sheep \( (F = 2.225, \ p = .041) \).

In separate experiments, unlabeled IGF-II significantly inhibited the \( V_d \) at the CP but not in the EP and CX. At the CP, unlabeled IGF-II reduced \( V_d \) by 34%–40% \( (p < .05) \) in both young and old tissues.

**Effect of IGFBP-2 and BSA on IGF-II Uptake**

IGFBP-2 at the lowest concentrations used (0.01 and 0.1 \( \mu \text{g/mL} \)) enhanced IGF-II uptake in young CP, but not in old CP or in other brain tissues. There were significant differences in IGF-II uptake between young and old CPs at...
these concentrations (Figure 5). At supraphysiological concentrations of IGFBP-2 (1 μg/mL), the young CP uptake was suppressed to levels seen in the old tissue. As a control for nonspecific protein effects, BSA was added at 0.01 and 1 μg/mL and had no significant effect on 125I-IGF-II Vd for the young CP. At BSA concentrations close to those found for albumin in CSF (250 and 400 μg/mL), Vd was reduced in both old and young CP, but not in other tissues (Figure 6).

**DISCUSSION**

The CPs forming the blood–CSF barrier avidly secrete CSF containing a wide range of growth factors in brain, e.g., transforming growth factor-β, vascular endothelial growth factor, IGF-II, and IGFBPs (32), contributing to homeostasis throughout the central nervous system (CNS; 33). Changes have been demonstrated in human, rat, and sheep CP morphology and function (34,35) with increasing age, but little is known about IGF-II interaction with CP. To the best of our knowledge, this is the first study to compare CP IGF-II gene expression in young and old sheep using real-time PCR. We find that there is no age-related difference in IGF-II gene expression in the ovine CP, which is a positive aspect of aging CP function. Previous studies on IGF-II expression in old rat brain have generated conflicting results. Park and Buetow (36) found slight, but not significant, declines in whole rat brain IGF-II mRNA from young adulthood to senescence. The method used by Park and Buetow (36) presumably included the CP and meninges, because these tissues were not mentioned as being dissected out. Using the same method (Northern blot analysis) in the rat, Kitraki and colleagues (37) found a significant age-related decrease in IGF-II expression in discrete brain regions (HP, hypothalamus, striatum, cerebral cortex and cerebellum), which excluded CP and meninges. However, despite the maintenance of mRNA expression in sheep CP, translational deficiencies in the CP cannot be ruled out because the level of IGF-II protein in CSF (but not plasma) decreased with age.

Because the circulating IGFs may have therapeutic roles in neurodegenerative diseases, including AD (23), interest has recently been focused on the uptake of IGFs from the systemic circulation into the CSF compartment across brain barriers. Several studies in rat indicated that the entry of both 125I-IGF-I and -II from blood to brain (38), and 125I-IGF-I from blood to CSF, are saturable (39). Consistent with these studies, we found that uptake of 125I-IGF-II into CP from blood side was also saturable. The Km of 11–15 nM is indicative of a high-affinity transport system compared to published serum levels (200–500 ng/mL in sheep and up to 1000 ng/mL in humans, i.e., 25–140 nM) (40,41), similar to IGF-I uptake into CSF in the rat (39). The contribution of circulating IGF-II to newly formed CSF levels can be estimated from our kinetic data and CSF secretion rates in this preparation (29): Assuming IGF-II plasma concentrations of 7 nM (50 ng/mL, Figure 1), average flux into CP would be 2.9 pmol/min/g. CSF secretion rate averaged 97.3 μL/min/g; therefore, IGF-II in newly formed CSF could reach 30 nM (225 ng/mL), which is sufficient to account for normal CSF levels. It is of interest that IGF-II

<table>
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<th>Parameters</th>
<th>Young Sheep (N = 3)</th>
<th>Old Sheep (N = 4)</th>
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<tr>
<td>Umax, %</td>
<td>22.68 ± 5.48</td>
<td>23.40 ± 6.47</td>
</tr>
<tr>
<td>Un, %</td>
<td>18.72 ± 3.12</td>
<td>16.55 ± 2.98</td>
</tr>
<tr>
<td>Backflux, %</td>
<td>22.43 ± 3.94</td>
<td>28.20 ± 5.16</td>
</tr>
<tr>
<td>Vmax, pmol/min/g</td>
<td>9.52 ± 1.83</td>
<td>7.60 ± 0.79</td>
</tr>
<tr>
<td>Km, nm</td>
<td>15.27 ± 6.20</td>
<td>11.11 ± 3.79</td>
</tr>
<tr>
<td>Kd, mL/min/g</td>
<td>−0.03 ± 0.07</td>
<td>−0.10 ± 0.13</td>
</tr>
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Note: IGF-II = insulin-like growth factor 2; Umax = maximum cellular uptake; Un = average uptake; Vmax = maximum flux; Km = half-saturation constant; Kd = cellular diffusion constant.
IGF-II uptake into CP

uptake from the blood side of the CP did not require the presence of IGFBPs; this again is consistent with the study in rats (39). Comparisons between young and old sheep revealed no significant differences in kinetic constants, and $K_d$ was close to zero in both groups, indicating no detectable IGF-II passive diffusion into CP from the blood side.

When in CSF, IGF-II must then enter brain tissue to exert its effects. After incubation of brain tissues in aCSF with $^{125}$I-IGF-II, all studied regions demonstrated IGF-II sequestration (after correcting for extracellular space with mannitol), with no difference in uptake between those anatomically close to ventricular CSF (EP and HP) and the more distant cortical region (CX). Unlabeled IGF-II significantly inhibited $^{125}$I-IGF-II uptake into the CP, consistent with a receptor-mediated process, but no effect was seen in EP, HP, or CX, suggesting uptake via a nonreceptor-mediated route and/or less receptor at these tissues. The EP lining of choroid plexus and ependymal layer; CX = cortex; HP = hippocampus. Values are mean ± standard error of the mean, $n=4$. *p < .05, old compared to young.

Figure 3. Concentration of insulin-like growth factor II (IGF-II; both labeled and unlabeled) versus $^{125}$I-IGF-II flux into choroid plexus (CP) from the blood side in the in situ perfused young (filled squares) and old (open squares) CP. Values are mean ± standard error of the mean, $n=3$–4 for each point.

Figure 4. $^{125}$I-insulin-like growth factor II (IGF-II) $V_d$ (µL/mg) in isolated incubated young (filled bars) and old (open bars) brain tissues. CP = choroid plexus; EP = ependymal layer; CX = cortex; HP = hippocampus. Values are mean ± standard error of the mean, $n=4$. *p < .05, old compared to young.

(i.e., an autocrine response) or in bulk CSF. Uptake at the blood face would be by both vascular endothelium and the basolateral face of the CP epithelium. Although current data are lacking about the exact localization of receptor types at this face of the tissue, early studies on rat suggest a relatively high density of IGFR-2 at the vascular endothelium as well as presence at the epithelium (49), which targets IGF-II for internalization and degradation. Therefore, the consequences of IGF-II uptake may be different at the two faces of the CP. With increasing age, IGFR-1 function is seen to decline in many systems—for example, in the human eye lens (50)—and may be associated with normal aging throughout the body (51), including the CP. Recent work has suggested that blocking IGF-1 receptor function specifically in the CP exacerbates AD pathology in a mouse model with cerebral amyloidosis (51), highlighting the importance of the CP in IGF homeostasis and CNS health. It is not clear what causes age-related loss of function, but at the CP it is likely that loss of apical surface area due to microvilli flattening (52) contributes to reduced uptake at the CSF face when compared to the blood face. In addition, oxidative stress associated with aging may reduce receptor affinity (53), but it is unknown whether this would affect one receptor type more than any other.

IGF-II is known to be bound to proteins in both serum and CSF (1). Albumin, the most abundant protein in CSF (32), significantly inhibited $^{125}$I-IGF-II $V_d$ at the CP at physiological concentrations. Albumin can bind to a number of hormones (e.g., thyroxine, aldosterone, cortisol, progesterone) to reduce oscillating levels of free hormone and to transport steroid hormones (54). Although there is no report of IGFs binding to albumin (54), our results suggest that BSA does interact with IGF-II or its binding sites, to reduce $V_d$ at the CP. Our previous study using this method (55) demonstrated that BSA at 0.4 mg/mL significantly inhibited thyroxine ($T_d$) uptake, not only at the CP but also in other brain regions, and to a greater extent than for IGF-II, suggesting that BSA has less affinity for IGF-II compared to $T_d$.

Of the specific IGF binding proteins in CNS, IGFBP-2, -4, -5, and -6 mRNA expression has been demonstrated in the CP and meninges of the ventricular system, with IGFBP-2 predominating in rat (15,56). Some IGFBPs (-2, -5, -6) bind with preferential affinity to IGF-II, but none preferentially binds to IGF-I (57). Among them, IGFBP-2 appears to be the major component in CSF and mammalian brain...
IGFBP-2 and IGF-II were found to have "identical" anatomical and cellular distributions in the adult rat brain (56), suggesting that IGFBP-2 modulates IGF-II function in brain (15). The functions of IGFBPs in the CSF have been suggested to be transport, buffering, and targeting of IGFs between sites of biosynthesis and activity (5). However, Pulford and Ishii (39) argued that IGF influx from blood to CSF was independent of IGF receptors and IGFBPs, although IGFBPs prolong half-lives and regulate biological activities of IGFs. Our study demonstrated that, although IGFBP-2 was not necessary for $^{125}$I-IGF-II uptake, it did enhance uptake into the CP from aCSF at low concentrations. The increase of $^{125}$I-IGF-II uptake with IGFBP-2 was only seen in the young tissue, indicating a deficit with age. The role of IGFBP-2 in modulating IGF-II receptor interaction is an ongoing area of research. In general, soluble IGFBP-2 is considered to bind IGF-II in the medium (4,58).
preventing IGF-II interacting with IGFR-1 and thus having an inhibitory effect on IGF-II action (59). However, IGFBP-2 in the presence of IGF-II can also interact with cell surface proteoglycan binding sites and glycosaminoglycans (60), leading to membrane-associated complexes that retain affinity for IGF-II (61). The consequences for cell function are unclear, but Firth and Baxter (59) suggest that this would concentrate IGFs near IGFR-1, potentiating their effects. Whichever effect of IGFBP-2 predominates (potentiating or inhibitory) may depend on the relative concentrations of both IGFBP and membrane binding sites. In our incubation studies with the young CP (Figure 6), we could envisage that at low IGFBP-2 concentrations the binding protein is associated with membrane sites and concentrates IGF-II near its receptors for uptake. At higher IGFBP-2 levels where membrane sites are saturated, soluble IGFBP-2 would increasingly bind IGF-II in the medium, preventing access to the receptors and reducing uptake. Further studies quantifying potential membrane and receptor binding site densities would be needed to test this hypothesis.

The expression of IGF-II and IGFBP-2 in the sheep CP is maintained throughout development and into adulthood, and Delhanty and Han (62) suggest that this dual, high level of expression ensures that growth and development occur in a coordinated and controlled way. Indeed, a synergistic relationship between IGF-II and IGFBP-2 expression has been suggested by Reijnders and colleagues (63) in a transgenic mouse model overexpressing human IGF-II, which also upregulates IGFBP-2 expression in medulla. The CP retains dual expression into adulthood, potentially acting as both a production and degradation site for IGF, depending on the needs of the CNS, and helping to maintain CNS homeostasis in health and disease. A vital part of this homeostasis would be targeted removal of IGF due to CP uptake or binding, to deplete CSF levels when required (via IGFR-2), and/or to maintain function of the CP itself in an autocrine manner (via IGFR-1). The decline in IGF-II uptake and IGFBP-2 modulation with old age could affect both homeostasis of IGF-II in CSF and brain extracellular fluid and the positive feedback on CP maintenance itself.

Conclusion
We have found evidence for the CP–CSF system playing a significant role in maintenance of CSF IGF-II, and thus in delivery of IGF-II to brain. Although there was no age-related change in mRNA expression for IGF-II, IGF-II protein levels decreased with age. Normal aging diminished both the baseline uptake of IGF-II at the CSF face of the CP and the enhanced uptake in the presence of IGFBP-2. Whether this reflects a reduction in IGFR-1 is not known, but the result would be reduced paracrine/autocrine effects of IGF-II on the CP with implications for CNS health.

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References

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