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Prenatal dynamics of kynurenine pathway metabolism in mice: focus on kynurenic acid

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Abstract

The kynurenine pathway (KP), the major catabolic route of tryptophan in mammals, contains several neuroactive metabolites, including kynurenic acid (KYNA) and 3-hydroxykynurenine (3-HK). KP metabolism, and especially the fate of KYNA, during pregnancy is poorly understood, yet may play a significant role in the development of psychiatric disorders later in life. The present study was designed to investigate the prenatal features of KP metabolism *in vivo,* with special focus on KYNA. To this end, pregnant CD-1 mice were treated systemically with kynurenine (100 mg/kg), KYNA (10 mg/kg) or saline at embryonic day 18. As expected, administration of either kynurenine or KYNA increased KYNA levels in maternal plasma and placenta. Maternal kynurenine treatment also raised kynurenine levels in fetal plasma and brain, demonstrating the ability of this pivotal KP metabolite to cross the placenta, and increase the levels of both KYNA and 3-HK in the fetal brain. In contrast, maternal administration of KYNA caused only a small, non-significant elevation of KYNA levels in fetal plasma and brain. Complementary experiments using an *ex vivo* placental perfusion procedure confirmed the significant transplacental transfer of kynurenine, and demonstrated that only a very small fraction of maternal kynurenine is converted to KYNA in the placenta and released into the fetal compartment under physiological conditions. Jointly, these results begin to clarify the contributions of the maternal circulation and the placenta to fetal KYNA in the late prenatal period.

Keywords

3-Hydroxykynurenine; Neurodevelopment; Placenta; Schizophrenia

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Introduction

The kynurenine pathway (KP) constitutes the main catabolic route of the essential amino acid tryptophan in mammals. The pivotal metabolite of the pathway, L-kynurenine ("kynurenine"), is the direct bioprecursor of two neuroactive compounds, kynurenic acid (KYNA) and 3-hydroxykynurenine (3-HK). KYNA, a neuroinhibitory compound with neuroprotective properties [1,2], is formed by irreversible transamination of kynurenine, whereas the production of 3-HK, a free radical generator with pro-excitotoxic features [3], is catalyzed by kynurenine 3-monooxygenase (KMO) in a separate branch of the pathway (Fig. 1) [4,5].

Malfunction of the KP has been linked to the pathophysiology of several psychiatric disorders, including schizophrenia (SZ). In SZ, elevated levels of KYNA are found in postmortem brain and cerebrospinal fluid [6-9] and may be causally related especially to the cognitive deficits observed in this disorder. Experimentally, in line with the neurodevelopmental hypothesis of SZ etiology [10-12], increased levels of KYNA in the fetal rat brain cause an elevation of brain KYNA and cognitive impairments in adulthood [13-16]. Notably, cerebral 3-HK levels do not appear to be abnormal in SZ [6] and also remain unchanged in adulthood following perinatal KP manipulation [13]. Although KYNA and other KP metabolites are neuroactive and may influence fetal neurodevelopment [14], little is known about their production and regulation during pregnancy.

In the mammalian brain, the levels of kynurenine, KYNA and 3-HK are normally considerably higher prenatally than after birth, and the KYNA-synthesizing enzymes kynurenine aminotransferase (KAT) I and II, as well as KMO, are present in the fetal brain [17-20]. The placenta, which connects the maternal and fetal blood circulations, also expresses KP enzymes, including KATs and KMO [21-24], and recent studies in mice have also ascertained the presence of KYNA and 3-HK in this tissue [25,26]. Notably, the placenta, which allows nutrient transport and is critical for fetal growth and development [27], not only converts maternally derived tryptophan to serotonin during the early periods of pregnancy, providing an exogenous source of the neurotransmitter to the fetus [28], but also transforms tryptophan to kynurenine, as shown in studies using cultured human placental explants [22]. This raises the possibility that KP metabolites in the fetus, and specifically in the fetal brain, derive from the placenta. Whether maternal kynurenine and its metabolites enter the fetus by diffusion or specific transport mechanisms, whether kynurenine is degraded in the placenta and its metabolites are then released into the fetal compartment, and whether externally derived kynurenine stimulates KP metabolism mainly in the fetus itself, remains unknown, however. In other words, the dynamics of the KP during the prenatal period, and in particular the effect of maternal kynurenine fluctuations on the fetus, are still poorly understood.

The present study was designed to fill this void by investigating KP metabolism during pregnancy in greater detail. Although we also measured 3-HK levels in parallel in most experiments, we focused primarily on the disposition of KYNA, because it can inhibit NMDA and α7 nicotinic acetylcholine receptors, which are both critically involved in brain development [29,30]. In the first set of experiments, kynurenine, KYNA or saline were

orally administered to pregnant CD-1 mice at embryonic day (ED) 18, and analytes were measured in maternal plasma, placenta, fetal plasma and fetal brain. In complementary experiments, we studied the role of the placenta in KP metabolism directly by using an established *ex vivo* placental perfusion technology [31].

Materials and Methods

Chemicals

KYNA, 3-hydroxy-DL-kynurenine (3-HK), bovine serum albumin (BSA), Fast Green FCF and antipyrine were obtained from Sigma-Aldrich (St. Louis, MO, USA). L-Kynurenine sulfate ("kynurenine"; purity: 99.4%) was obtained from Sai Advantium (Hyderabad, India). Dulbecco's Modified Eagle Medium (DMEM) was purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). All other biochemicals and chemicals were obtained from various commercial suppliers and were of the highest available purity.

Animals

For *in vivo* experiments (performed at the University of Maryland), timed-pregnant CD-1 dams were obtained from Charles River Laboratories at ED2. The mice were maintained on a 12 h/12 h light/dark cycle in a temperature-controlled room, with food and water provided *ad libitum.* Three embryos per litter were used for the analysis of KP metabolites, and the data were expressed as an average of litters.

For *ex vivo* experiments (performed at the University of Southern California), timed-pregnant CD-1 dams were purchased from Charles River Laboratories at ED12 and maintained on a 12 h/12 h light/dark cycle in a temperature-controlled room, with food and water provided *ad libitum.* At ED18, dams were anesthetized via isoflurane inhalation, and a single placenta per animal was harvested as previously described (Bonnin et al., 2011; Goeden and Bonnin, 2012).

All experimental animals were housed in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. All procedures involving animals were approved by the local Institutional Animal Care and Use Committee of the investigators' institutions (University of Maryland Baltimore and University of Southern California, respectively).

Kynurenine and KYNA treatment *in vivo*

At ED18, kynurenine (100 mg/kg), KYNA (10 mg/kg) or sterile saline were administered orally to pregnant dams (n=3 per group). Based on the literature [26,32,33] and pilot data (not shown), which consistently demonstrated very rapid effects of various experimental interventions on KP metabolites in dams and neonates, the mice were euthanized 90 min later using CO_2, and maternal and fetal plasma (supernatant of blood centrifuged at 5,200 × g, 10 min), placenta and fetal brain were collected and stored at −80°C until analysis.

Ex vivo placental perfusion assay

A single placenta per dam (n=4-7 per experimental group) was harvested and quickly transferred to a thermostatically controlled incubation chamber at 37°C. The uterine artery was cannulated with a 200 μm diameter catheter, and perfused at 20 μL/min with DMEM containing bovine serum albumin (BSA; 100 mg/mL) alone or in combination with 100 μM kynurenine for both transfer and metabolism experiments. All simulated maternal solutions contained 100 μM antipyrine, a passive diffusion marker used as positive control and an index of perfusion efficiency [34]. The umbilical artery was cannulated with a 105 μm diameter catheter and perfused at 5 μL/min with DMEM and 0.01% Fast Green FCF. The eluate was collected from the umbilical vein for 120 min at 10 min intervals, and analyzed for kynurenine and KYNA content (see below). The transplacental transfer rate (TPT%) of kynurenine was determined using the equation: TPT% = (Cf × Sf × 100)/(Cm × Sm) [Cf: fetal concentration (measured), Cm: maternal concentration (input, measured); maternal flow (Sm) 20 μL/min, fetal flow (Sf): 5 μL/min; see Goeden and Bonnin, 2013, for detailed methodology].

Kynurenine and KYNA measurement

The tissue was weighed while frozen. After thawing, fetal brain (1:10, w/v) and placenta (1:10, w/v) were homogenized by sonication in ultrapure water (placental homogenate was further diluted 1:10, v/v, for KYNA determination). Maternal (1:4, v/v for kynurenine, 1:10, v/v for KYNA) and fetal (1:5, v/v for kynurenine, 1:20 v/v for KYNA) plasma were diluted in ultrapure water. Twenty-five μL of 25% perchloric acid were added to 100 μL of the tissue preparation, and precipitated proteins were removed by centrifugation (16,000 × g, 15 min). For determination *ex vivo,* the eluate was diluted 1:2 (v/v) in 6% perchloric acid and centrifuged (16,000 × g, 5 min).

For kynurenine and KYNA determination, 20 μL of each resulting supernatant were injected onto a 3 μm C18 reverse phase HPLC column (150 mm × 4 mm; Dr. Maisch GmbH, Ammerbuch, Germany), using a mobile phase containing 50 mM sodium acetate and 3% acetonitrile (pH adjusted to 6.2 with glacial acetic acid) at a flow rate of 0.5 mL/min. Zinc acetate (0.5 M; not pH adjusted), was delivered post column by a peristaltic pump (Dionex AXP, Thermo Fisher, Waltham, MA, USA) at a flow rate of 0.1 mL/min. In the eluate, analytes were detected fluorimetrically (kynurenine: excitation: 365 nm, emission: 480 nm; KYNA: excitation: 344 nm, emission: 398 nm; S200a fluorescence detector; Perkin Elmer, Waltham, MA, USA).

3-HK determination

Fetal brain (1:10, w/v) and placenta (1:20, w/v) were homogenized, and maternal (1:2, v/v) and fetal plasma (1:4, v/v) were diluted, in ultrapure water. Twenty-five μL of 25% perchloric acid were then added to 100 μL of each preparation, and precipitated proteins were removed by centrifugation (16,000 × g, 15 min). Twenty μL of the resulting supernatant were then injected onto a 3 μM HPLC column (80 mm × 4.6 mm; Thermo Fisher Scientific, Waltham, MA, USA), using a mobile phase consisting of 1.5% acetonitrile, 0.9% triethylamine, 0.59% phosphoric acid, 0.27 mM EDTA and 8.9 mM sodium heptane sulfonic acid, and a flow rate of 0.5 mL/min. In the eluate, 3-HK was

detected electrochemically using an HTEC 500 detector (Eicom, San Diego, CA, USA; oxidation potential: + 0.5 V).

Antipyrine detection

Following extraction for kynurenine and KYNA quantification via HPLC, antipyrine concentrations in maternal input solutions and fetal eluates were assessed with the use of a spectrophotometer (Implen, Westlake Village, CA, USA). To avoid confounding background peaks from the perfusion media, antipyrine standard curve solutions were diluted in the fetal input solution, and extracted in the same way as the fetal eluate samples prior to HPLC analysis. The antipyrine concentration in the samples was then quantified by measuring absorption at 240 nm, as previously described [35].

Protein measurement

Proteins were determined according to the method of Lowry et al. (1951) [36], using BSA as a standard.

Statistical analysis

Results are expressed as the mean ± SEM. One-way Anova followed by Bonferroni's post-hoc test or Mann-Whitney U test were used to determine statistical significance in all experiments. A p value of <0.05 was considered significant.

Results

To gain insight into the disposition of kynurenine and KYNA in the late prenatal period, we administered these two compounds separately to pregnant mice at ED18 and then determined the levels of the two KP metabolites, as well as 3-HK levels, in maternal and fetal tissues.

KP metabolite levels in maternal plasma and placenta

First, we measured the analytes in the maternal plasma. As expected, kynurenine was significantly elevated 90 min after its administration (Fig. 2A), compared to mice treated with either vehicle or KYNA ($p<0.01$). KYNA and 3-HK levels were also increased, by 6- and 15-fold, respectively, after kynurenine administration (Fig. 3A, 4A). Again as anticipated, KYNA, too, was recovered from the blood following its oral administration (Fig. 3A), and no changes in kynurenine or 3-HK levels were observed (Fig. 2A, 4A). Notably, the administration of 10 mg/kg KYNA resulted in approximately 6 times higher circulating KYNA levels than treatment with 100 mg/kg kynurenine ($p<0.01$).

As in the maternal plasma, maternal administration of kynurenine also raised the levels of all metabolites analyzed in the placenta (Fig. 2B, 3B, 4B). Kynurenine levels increased 9-fold, while KYNA and 3-HK levels were 3- and 15-fold higher, respectively, than in controls. As in the plasma, maternal treatment with KYNA significantly increased KYNA levels in the placenta (Fig. 3B), but had no effect on either kynurenine or 3-HK levels (Fig. 2B, 4B).

Jointly, these results confirmed that maternal administration of either kynurenine or KYNA induces an increase in KYNA levels in maternal plasma and placenta.

KP metabolite levels in fetal plasma and brain

Next, we analyzed KP metabolites in the fetus after oral administration of kynurenine or KYNA. Compared to vehicle, kynurenine treatment caused a significant increase in the levels of kynurenine ($p<0.001$), KYNA ($p<0.01$) and 3-HK ($p<0.05$) in the fetal plasma (Fig. 2C, 3C, 4C). However, we did not detect an increase in KYNA levels after its maternal administration (Fig. 3C), nor did KYNA treatment affect kynurenine or 3-HK levels in fetal plasma (Fig. 2C, 4C).

Kynurenine administration to the dam significantly elevated the levels of all metabolites analyzed in the fetal brain (Fig. 2D, 3D, 4D). Specifically, kynurenine was 10-fold higher than control levels (Fig. 2D), while KYNA and 3-HK increased by 5- and 17-fold, respectively (Fig. 3D, 4D). In contrast, as in the fetal plasma, oral KYNA administration to the dam did not significantly affect the levels of any of the three compounds in the fetal brain.

Transplacental transfer of kynurenine

An *ex vivo* perfusion paradigm was used to investigate the involvement of the placenta in the movement of kynurenine from mother to fetus. To this end, a single placenta per pregnant dam was harvested at ED18 and perfused with a) artificial medium (DMEM + BSA + 100 μM antipyrine) containing a near physiological concentration of kynurenine (~0.5 μM; control) or b) the same medium spiked with 100 μM kynurenine. In controls, we detected an average output of 1.2 ± 0.3 pmoles kynurenine/μL over the 120 min perfusion period (Fig. 5A). Normalized to flow rates and antipyrine transfer, these values corresponded to a TPT $\%^{KYN}$ rate of 43.6 ± 10.4% (Fig. 5B). When the maternal input concentration of kynurenine was raised to 100 μM, the average output concentration of kynurenine over the 120 min perfusion period increased to 3.8 ± 0.6 pmoles/μL, although with a greatly reduced rate of transplacental transfer ($TPT\%^{KYN}$ = 1.5 ± 0.2%; Mann-Whitney $U = 0$, $n_1 = 7$, $N_2 = 4$, $p = 0.0095$). Of note, the TPT% of antipyrine was comparable between individual perfusions and perfusion conditions (on average, $TPT\%^{Antipyrine}$ = 11.4 ± 0.9%), indicating similar intra- and inter-assay perfusion efficiencies.

These results confirmed that maternal kynurenine crosses the placenta, as observed *in vivo*.

Placental synthesis of KYNA

As our *in vivo* results showed that increased kynurenine concentrations in the maternal plasma cause KYNA levels to increase in fetal plasma and fetal brain (Fig. 3C, 3D), we also assessed whether KYNA was synthesized in, and subsequently released from, the placenta. The *ex vivo* perfusion system enabled us to test this possibility independent of maternal and fetal kynurenine to KYNA metabolism. To this end, we perfused ED18 placentas, as described above, with artificial medium (control) or with medium containing 100 μM kynurenine, using our *ex vivo* paradigm (Fig. 5C). With physiological levels of kynurenine, we detected an average output of 50.6 ± 7.0 fmoles KYNA/μL over the 120 min perfusion

period. Placental perfusion with 100 μM kynurenine induced only a small increase in KYNA in the fetal eluate (76.5 ± 8.4 fmoles/μL; Mann-Whitney U = 5, n_1 = 7, N_2 = 5, p = 0.0480).

These results showed that the placenta does not play a major role in the control of KYNA levels in the fetus.

Discussion

In the present study, we examined some of the prenatal dynamics of KP metabolism in mice. Using dams on ED18, we focused particularly on the fate of KYNA, which can function as an antagonist of NMDA and α7 nicotinic acetylcholine receptors, both of which play essential roles in brain development [29,30]. As illustrated schematically in Fig. 6, we found that KYNA can be readily recovered from the maternal circulation, the placenta and the fetus after systemic kynurenine administration to the dam. However, no increase in KYNA levels was observed in fetal plasma and brain after KYNA itself was given maternally, indicating that peripherally applied KYNA does not cross the placenta. In a complementary set of experiments, these *in vivo* results were evaluated in greater detail using an established *ex vivo* placental perfusion method.

The present demonstration that an experimentally induced, acute increase in kynurenine in the maternal blood promptly leads to substantial elevations in kynurenine levels in placenta, fetal plasma and fetal brain clearly indicated that kynurenine crosses from the mother to the placenta and reaches the fetus *in vivo.* Furthermore, we also observed higher concentrations of kynurenine, as well as KYNA and 3-HK, in fetal compared to maternal plasma in *vehicle-treated* animals, suggesting that transplacental transfer and fetal enrichment of these KP metabolites occurs normally during pregnancy. This conclusion is in excellent agreement with previous studies in both rodents and humans [23,37,38]. In marked contrast, systemic KYNA administration to the dam, while raising KYNA levels in the maternal plasma and in the placenta, failed to affect the levels of the metabolite in fetal plasma or brain, even though the dose of KYNA used in these experiments (10 mg/kg) was deliberately chosen to exceed by several fold the plasma concentration of KYNA reached after treatment with 100 mg/kg kynurenine (cf. Fig. 3A). These results suggest that KYNA enters the placenta from the maternal circulation, or is synthesized locally, but subsequently does not reach the fetus. It follows that transplacental KYNA transfer either does not occur at all or saturates at low KYNA concentrations. As the placenta is a highly vascularized organ [39], we also measured placental KYNA levels in separate mice, which were perfused with sterile saline to partially flush maternal blood from the organ. In these animals, we observed a 40% reduction in KYNA content compared to non-perfused placentas (n=4 per group; data not shown). Therefore, the increase in KYNA levels recovered from the placenta after its systemic administration to the dam *in vivo* appears to be, at least in part, due to the presence of residual maternal blood, which contained considerable amount of KYNA.

In order to investigate the respective roles of the various compartments further, we examined the transfer of kynurenine from the mother to the fetus using an *ex vivo* placental perfusion system [31]. Consistent with our *in vivo* findings, these experiments revealed that perfusion with kynurenine raised the fetal output concentration of kynurenine substantially, whereas

KYNA output levels increased only slightly. Interestingly, by comparing the effects of perfusions with low (i.e. essentially physiological) and high kynurenine, and by determining TPT%, we found that transplacental kynurenine transfer saturates as maternal concentrations of kynurenine reach high, hyperphysiological levels. These results confirmed that elevated maternal plasma kynurenine levels normally cause kynurenine concentrations to rapidly increase in the fetal plasma separate from *de novo* placental synthesis, and further suggest the presence of a carrier-mediated process, potentially similar to the amino acid transporter identified for tryptophan [40].

The *ex vivo* experiments were also designed to examine the role of the placenta in the *de novo* synthesis of KYNA from maternally derived kynurenine. In line with the results obtained *in vivo,* only a relatively small amount of KYNA was released from the isolated placenta upon perfusion with kynurenine. Although this study was unable to categorically differentiate between intraplacental synthesis and transplacental transfer of KYNA due to the presence of KYNA in the BSA that was added to the perfusion solution, these data demonstrated that the placenta normally plays at best a minor role in converting maternal kynurenine to KYNA. Therefore, besides limiting maternal-fetal KYNA transfer, the placenta does not appear directly involved in regulating KYNA levels in the fetus. This conclusion is in excellent agreement with the study of Manuelpillai et al. (2005), which reported very limited KYNA production from tryptophan in human placental extract under physiological conditions. Thus, the increase in fetal KYNA concentrations seen when kynurenine levels rise in the maternal plasma is likely due to KYNA neosynthesis in the fetus itself, rather than an upstream conversion of kynurenine (either maternal or placental). In line with these results, recent studies in tissue slices demonstrated that the fetal mouse brain is indeed able to effectively synthesize KYNA from kynurenine [41].

Administration of kynurenine to the dam *in vivo* also led to increased concentrations of 3-HK in the maternal plasma, in the placenta, and in fetal plasma and brain. The accumulations of 3-HK in the maternal circulation and in the placenta were quantitatively similar to the elevations seen in the fetal compartments, paralleling the effects of systemic kynurenine application. As 3-HK, in contrast to KYNA, crosses the blood-brain barrier, using the same transporter as kynurenine (i.e. the large neutral amino acid transporter; [42], and considering the chemical similarity of the two compounds, it is therefore likely that newly produced 3-HK transfers from the maternal circulation to the fetus using the same mechanisms as kynurenine. Additionally, the increase in fetal 3-HK levels following maternal kynurenine administration is probably enhanced further by enzymatic 3-HK formation *within* the fetus. Finally, as shown here, neither the transplacental transfer of kynurenine and 3-HK nor the fetal production of 3-HK from kynurenine appears to be affected by KYNA, as previously observed in the brain of adult rodents [43]. We therefore conclude that the presence – and by implication the possible function – of 3-HK in each compartment depends essentially on the efficiency of KMO, its biosynthetic enzyme.

Notably, it may not be appropriate to extrapolate the present results and interpretations to circumstances that are considered to be outside the normal physiological range. Thus, circulating kynurenine levels fluctuate significantly under various pathological conditions [44-48]. Moreover, placental morphology, blood circulation and gene expression – and

therefore placental function – are significantly influenced by nutritional status, stress and immune activation, which, in turn, influence prenatal brain development (see [27,49] for reviews). Of special interest in the present context, these factors not only regulate the placental conversion of tryptophan to serotonin and therefore cerebral serotonin levels [50] but also cause rapid elevations of both kynurenine and KYNA levels in the fetal brain [26,51].

Jointly, these phenomena account for the fact that elevated levels of kynurenine in the maternal blood, effected prenatally by varying tryptophan availability [32,52,53], direct delivery [14,16], pharmacological intervention [54] or adverse pathological events (see above), lead to an increased presence of kynurenine, KYNA – and in some cases 3-HK – in fetal plasma and brain. Since KYNA and other KP metabolites are neuroactive, these changes may in turn influence fetal neurodevelopment [14]. These considerations may be particularly relevant for neurodevelopmental disorders, including SZ, in which the reduced expression and activity of KMO may lead to increased kynurenine availability in the mother and, consequently, in the fetus [7,55].

Taken together, the present experiments complement previous reports in rodents, which had shown that the feeding of pregnant dams with kynurenine during the final week of gestation causes elevations in both KYNA and 3-HK in the fetal brain [14,25], as well as increases in brain KYNA levels in adulthood [14]. Those studies, as well as conceptually similar experiments examining the effects of systemic administration of the KMO inhibitor Ro 61-8048, revealed detrimental long-term consequences of prenatal KP stimulation [56-58], but were not designed to differentiate whether the elevated KYNA levels in the fetal brain derived from the maternal circulation or the placenta, or were a consequence of enhanced *in situ* production from kynurenine *within* the fetus. The present work demonstrated not only that KYNA does not gain access to the fetus from the mother but that the placenta, too, is a poor source of fetal KYNA under physiological conditions. Notably, the fact that measurements of KYNA in the maternal circulation have little bearing on KYNA concentrations in the fetal brain has important implications for studies in pregnant animals and humans.

In summary, acute increases in kynurenine – but not KYNA – levels in the maternal blood rapidly raise KYNA and 3-HK concentrations in the fetal brain under physiological conditions. Studies currently in progress in our laboratories are designed to determine the possible effects of systemic treatment of pregnant dams with 3-HK and other KP metabolites on early brain development. Together with the present findings, these studies are not only of interest with regard to the possible physiological role of kynurenines *in utero* but can be expected to be especially relevant for conceptualizing the role of abnormal maternal KP metabolism in the etiology of various neurodevelopmentally defined brain disorders, including SZ and autism.

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Abbreviations:

KYNA	Kynurenic acid
3-HK	3-Hydroxykynurenine
KP	Kynurenine pathway
KMO	Kynurenine 3-monooxygenase
SZ	Schizophrenia
KAT	Kynurenine aminotransferase
ED	Embryonic day
TPT%	Transplacental transfer rate

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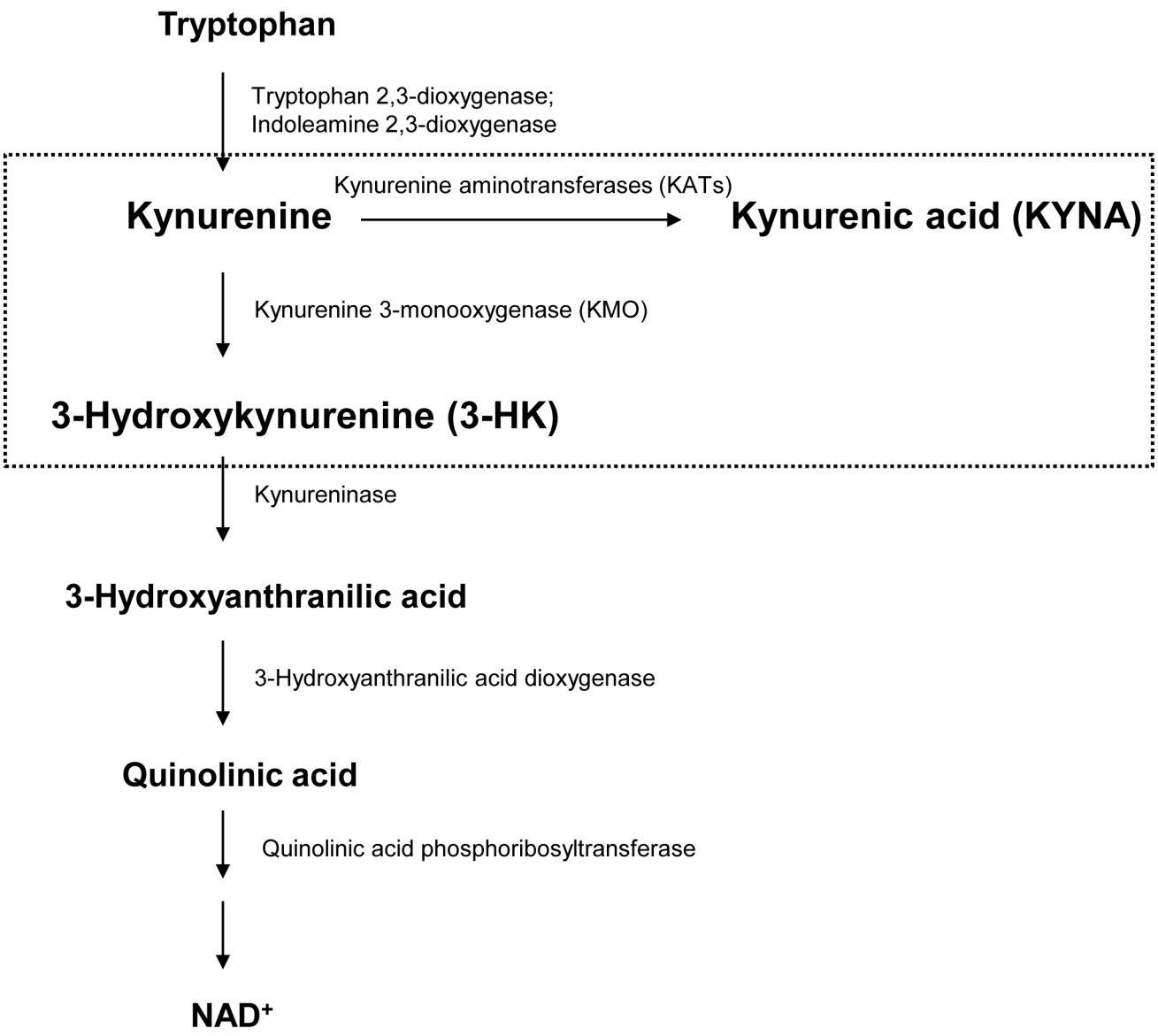

Figure 1:
The kynurenine pathway of tryptophan degradation.

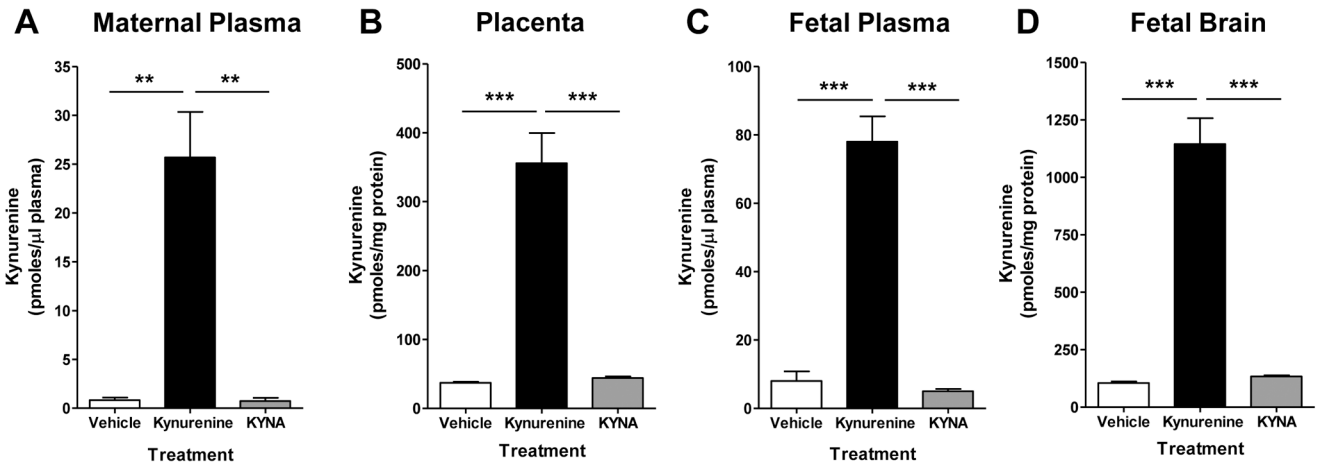

Figure 2:

Kynurenine levels in the maternal plasma (A), placenta (B), fetal plasma (C) and fetal brain (D) 90 minutes after oral treatment of pregnant CD-1 mice on ED18 with either saline (vehicle), kynurenine (100 mg/kg) or KYNA (10 mg/kg). Data are the mean ± SEM (n=3 dams per group; n=3 embryos per dam). **p<0.01, ***p<0.001 (one-way Anova, followed by Bonferroni's post-hoc test).

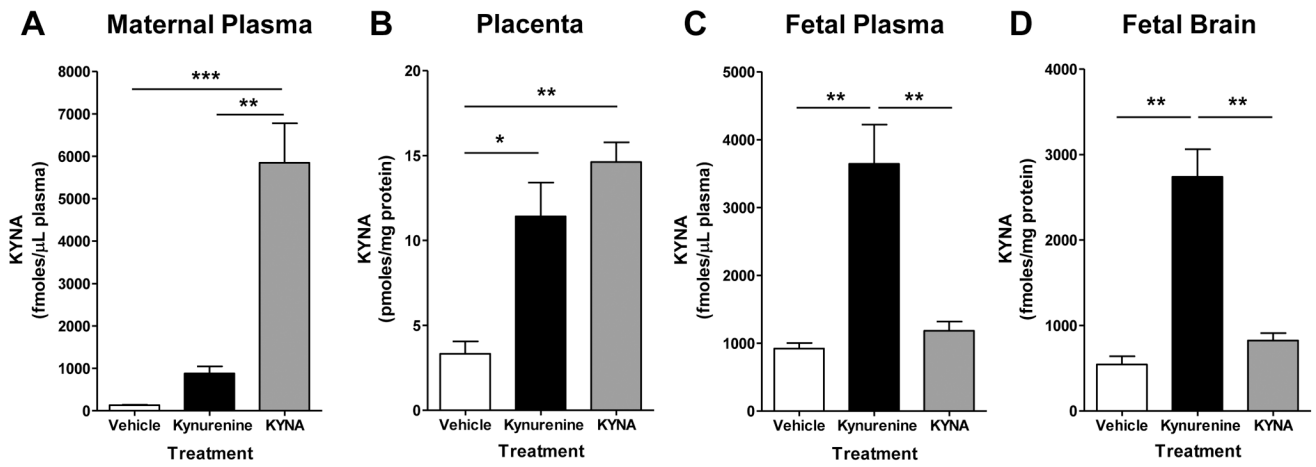

Figure 3:

KYNA levels in the maternal plasma (A), placenta (B), fetal plasma (C) and fetal brain (D) 90 minutes after oral treatment of pregnant CD-1 mice on ED18 with either saline (vehicle), kynurenine (100 mg/kg) or KYNA (10 mg/kg). Data are the mean ± SEM (n=3 dams per group; n=3 embryos per dam). *p<0.05, **p<0.01, ***p<0.001 (one-way Anova, followed by Bonferroni's post-hoc test).

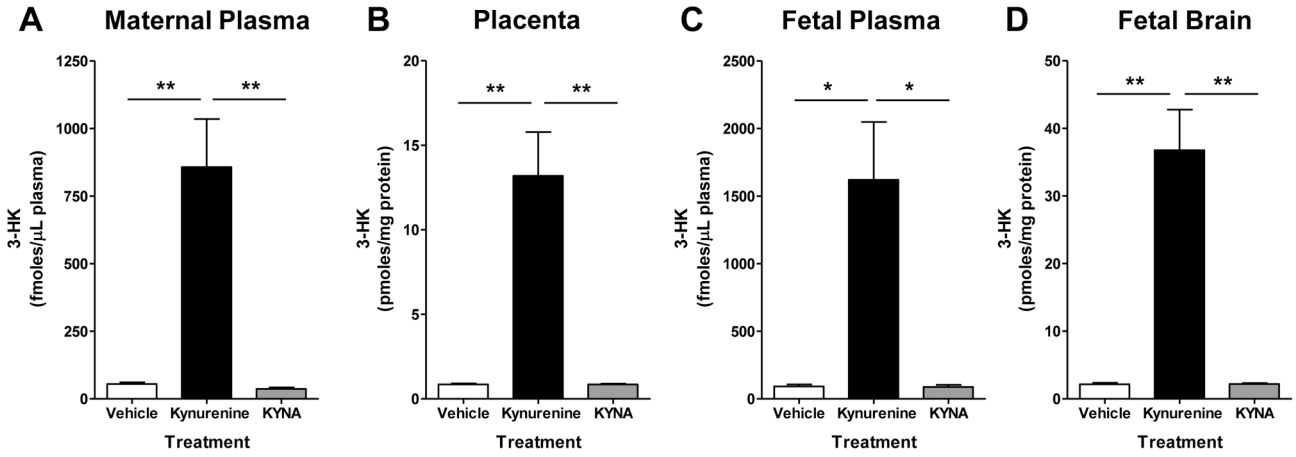

Figure 4:

3-HK levels in the maternal plasma (A), placenta (B), fetal plasma (C) and fetal brain (D) 90 minutes after oral treatment of pregnant CD-1 mice on ED18 with either saline (vehicle), kynurenine (100 mg/kg) or KYNA (10 mg/kg). Data are the mean ± SEM (n=3 dams per group; n=3 embryos per dam). *p<0.05, **p<0.01 (one-way Anova, followed by Bonferroni's post-hoc test).

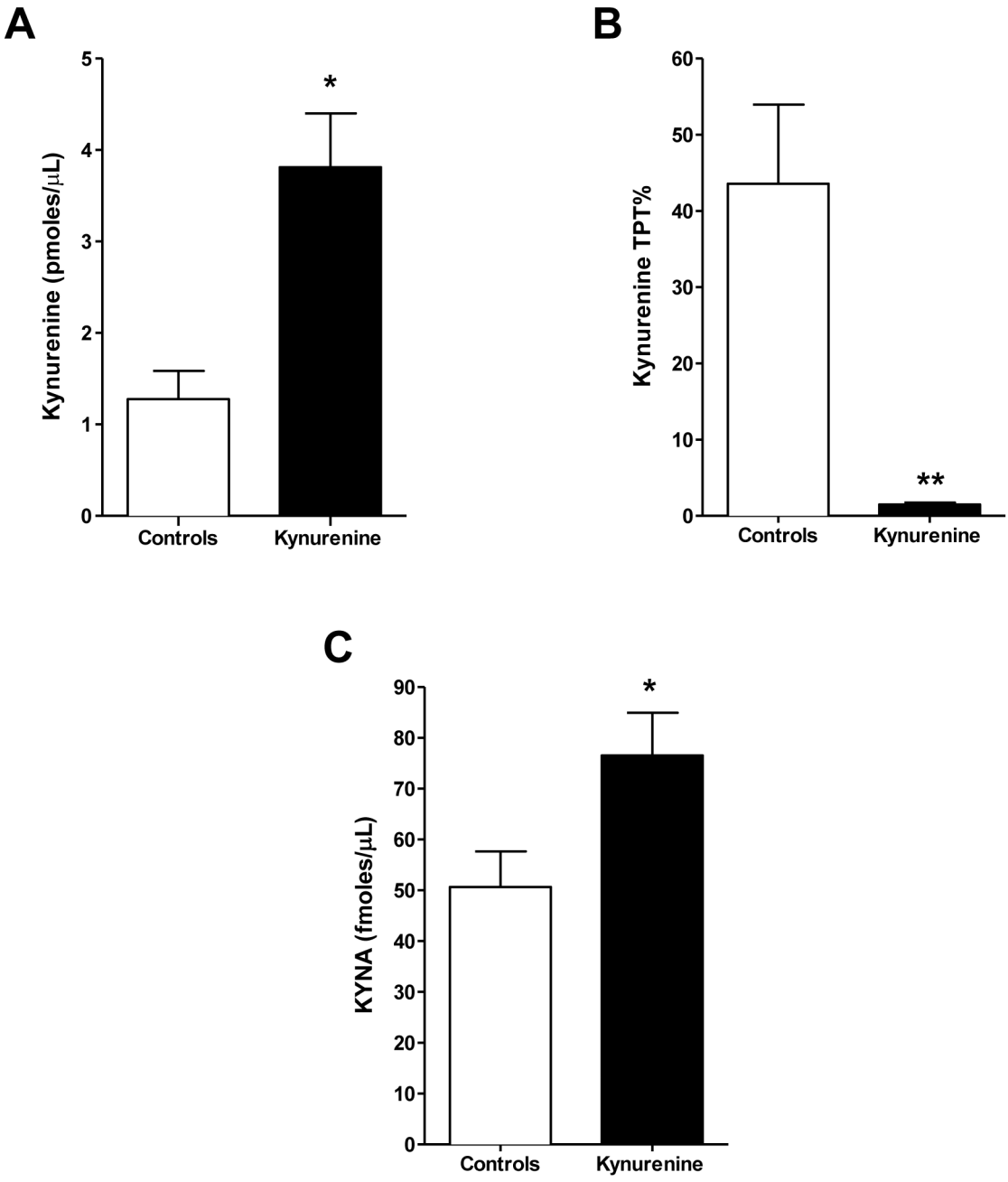

Figure 5:

Kynurenine transfer (A, B) through the placenta and KYNA production (C), determined *ex vivo* during a 120 min perfusion with a physiological concentration of kynurenine (0.5 μM; Controls) or 100 μM kynurenine (Kynurenine). Raw values shown in A and C were measured in the fetal eluate. See Materials and Methods for experimental details and definition of the transplacental transfer rate (TPT%). Data are the mean ± SEM (n=4-7 dams). *p<0.05, **p<0.01 (Mann-Whitney U test).

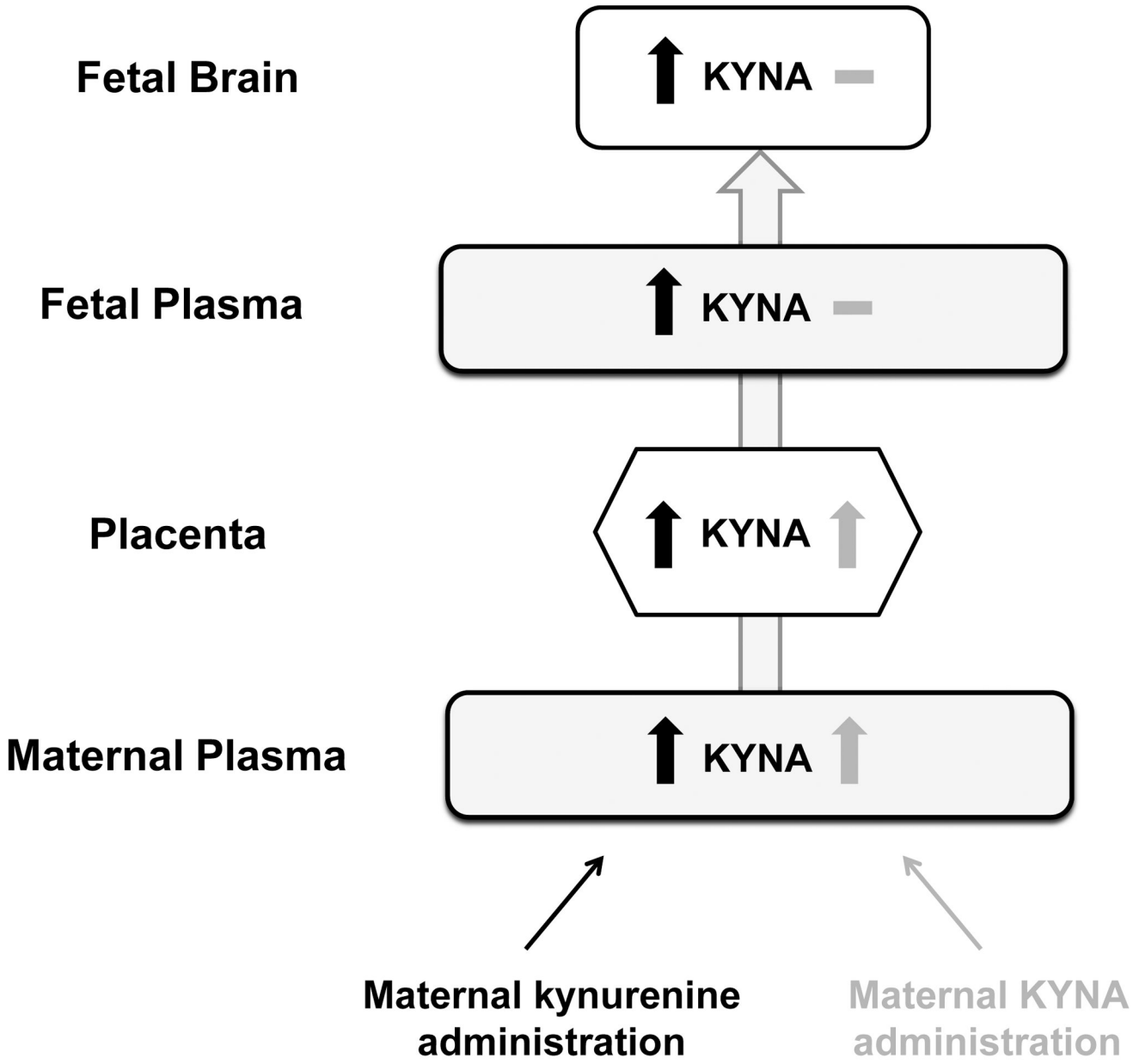

Figure 6:
Schematic illustration of KYNA content in various compartments after systemic administration of kynurenine or KYNA to the dam on ED18.