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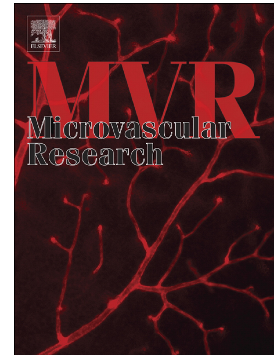

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Systemic administration of L-kynurenine sulfate induces cerebral hypoperfusion transients in adult C57Bl/6 mice.

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HIGHLIGHTS

1. I.p. administered L-kynurenine doesn't alter systemic blood pressure in anaesthetized mice.
2. I.p. administered L-kynurenine induces cerebral hypoperfusion transients in anaesthetized mice.
3. Kynurenic acid should be accounted for the observed dysregulation of the pial circulation.

KEYWORDS

L-Kynurenine; cerebral blood flow; laser speckle flowmetry; hypoperfusion; mean arterial blood pressure; C56Bl/6 mice;

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ABSTRACT

The kynurenine pathway is a cascade of enzymatic steps generating biologically active compounds. L-kynurenine (L-KYN) is a central metabolite of tryptophan degradation. In the mammalian brain, L-KYN is partly converted to kynurenic acid (KYNA), which exerts multiple effects on neurotransmission. Recently, L-KYN or one of its derivatives were attributed a direct role in the regulation of the systemic circulation. L-KYN dilates arterial blood vessels during sepsis in rats, while it increases cerebral blood flow (CBF) in awake rabbits. Therefore, we hypothesized that acute elevation of systemic L-KYN concentration may exert potential effects on mean arterial blood pressure (MABP) and on resting CBF in the mouse brain. C57Bl/6 male mice were anesthetized with isoflurane, and MABP was monitored in the femoral artery, while CBF was assessed through the intact parietal bone with the aid of laser speckle contrast imaging. L-KYN sulphate (L-KYNs) (300 mg/kg, i.p.) or vehicle was administered intraperitoneally. Subsequently, MABP and CBF were continuously monitored for 2.5 hrs. In the control group, MABP and CBF were stable (69±4mmHg and 100±5%, respectively) throughout the entire data acquisition period. In the L-KYNs-treated group, MABP was similar to that, of control group (73±6mmHg), while hypoperfusion transients of 22±6%, lasting 7±3min occurred in the cerebral cortex over the first 60-120 min following drug administration. In conclusion, the systemic high-dose of L-KYNs treatment destabilizes resting CBF by inducing a number of transient hypoperfusion events. This observation indicates the careful consideration of the dose of L-KYN administration by interpreting the effect of kynurenergic manipulation on brain function. By planning clinical trials basing on kynurenergic manipulation possible vascular side effects should also be considered.

ABBREVATIONS

L-KYN, L-Kynurenine; KYNA, Kynurenic acid; α7nACh, α7 nicotinic acetylcholine; GPR35, G-protein-coupled receptor 35; NMDA, N-methyl-D-aspartate; L-KYNs, L-Kynurenine sulfate; CBF, cerebral blood flow; MABP, mean arterial blood pressure; $rCBF_x$, cerebrovascular autoregulatory index; α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), eNOS, endothelial nitric oxide synthase; nitric oxide synthase (NOS); neuropeptide Y (NPY)

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INTRODUCTION

L-kynurenine (L-KYN) is a central metabolite of tryptophan degradation: known as kynurenine pathway, it is a cascade of enzymatic steps generating biologically active compounds (Vécsei et al., 2013). Growth in the level of systemic L-KYN is particularly associated with a dose-dependent increase of its direct downstream metabolite kynurenic acid (KYNA) in the central nervous system, and in the periphery (Swartz et al., 1990). Elevation of brain KYNA content is correlated with attenuation in the concentration of extracellular glutamate (Carpenedo et al., 2001), dopamine (Rassoulpour et al., 2005) and acetylcholine (Zmarowski et al., 2009) in distinct cortical and subcortical brain regions. KYNA influences neurotransmission through multiple pre-and postsynaptic pathways. KYNA directly attenuates neurotransmitter release, partly by inhibiting α7 nicotinic acetylcholine (α7nACh) receptor located on presynaptic terminals (Hilmas et al., 2001), and partly by stimulating G-protein-coupled receptor 35 (GPR35) localized on neurons and astrocytes (Alkondon et al., 2015; Berlinguer-Palmini et al., 2013; Guo et al., 2008). However, the significance of KYNA action on α7nACh receptor was queried recently (Albuquerque and Schwarcz, 2013). Nonetheless, KYNA hinders glutamatergic postsynaptic currents by competitive antagonism of N-methyl-D-aspartate (NMDA) receptor at its allosteric glycine binding site (Birch et al., 1988). Moreover, in the periphery and in the brain during neuroinflammation, KYNA promotes anti-inflammatory responses due to activation of aryl hydrocarbon receptor and GPR35 expressed by immune-cells (Moroni et al., 2012; Nguyen et al., 2010), as well as it presumably also modulates neuron survival through extrasynaptic NMDA receptor (Parsons and Raymond, 2014; Schwarcz, 2016). Besides its receptor-mediated actions, KYNA in itself is a potent antioxidant (Lugo-Huitrón et al., 2011).

Therefore, elevation of brain KYNA level, either by administration of L-KYN or pharmacological manipulation of the availability of the kynurenine pathway enzymes, has become an attractive strategy to attenuate neuroinflammatory responses and to protect against glutamate induced excitotoxicity associated with ischemic brain injury (Tan et al., 2012; Vécsei et al., 2013). Accordingly, we and our collaborators achieved neuroprotection by the administration of L-KYN sulfate (L-KYNs) in experimental models of neurodegenerative diseases and ischemic stroke (Gigler et al., 2007; Németh et al., 2004; Vámos et al., 2009). In these studies, neuroprotection was achieved partly by the administration of 300 mg/bwkg L-KYNs.

Acute or chronic elevation of systemic L-KYN content has already been suggested to trigger alteration in the behavior of rodents (Chess et al., 2007; Chess and Bucci, 2006;

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Vécsei and Beal, 1990; Wonodi and Schwarcz, 2010). Moreover, pre- and postnatal chronic L-KYN exposure in rodents provoked long-lasting neurochemical and behavioral abnormalities manifested in adulthood (Liu et al., 2014; Pocivavsek et al., 2014). We have shown formerly, that in naïve mice an acute administration of 300 mg/bwkg L-KYNs provoked hyperlocomotion and spatial working memory deficits, in conjunction with attenuated c-Fos protein expression in the striatum and the hippocampus (Varga et al., 2015). Accordingly, we have chosen the same dosage to obtain comparable information about the potential vascular effects of L-KYNs.

Recently, both L-KYN and KYNA were suggested to have some direct role in the regulation of the cardiorespiratory system. First, in the ventral part of the medulla and in the nucleus of the solitary tract many kynurenine aminotransferase-immunoreactive neurons were found in association with NMDA receptors involved in the control of blood pressure (Kapoor et al., 1997). Second, in a spontaneous hypertensive rat strain a missense mutation of kynurenine aminotransferase-I gene, a product of which converts L-KYN to KYNA, with abnormally low KYNA levels in the medulla were linked to the formation of systemic high blood pressure (Kapoor et al., 1994; Kwok et al., 2002). Yet, L-KYN administration dose dependently attenuated the elevated blood pressure (Wang et al., 2010). Third, L-KYN was identified as an endothelium-derived vasodilator, contributing to peripheral arterial relaxation and regulation of blood pressure during systemic inflammation in rats (Wang et al., 2010). Furthermore, an intravenous administration of low-dose L-KYN (1mg/kg) has been shown to increase cerebral blood flow (CBF) in conscious rabbits, mediated by activation of cholinergic and nitric oxide pathways (Sas et al., 2003). Moreover, kynurenine aminotransferase-immunopositive astrocytes and interneurons in the cerebral cortex and in distinct subcortical areas are in an ideal anatomical position to control local CBF (Guidetti et al., 2007; Herédi et al., 2016). Finally, region specific CBF reductions can be seen in schizophrenic patient suffering psychotomimetic distortions (Liddle et al., 1992; Mathew et al., 1982), whereas the cerebrospinal fluid content of L-KYN and KYNA are significantly elevated (Schwarcz et al., 2001).

Taken together, we hypothesized that acute elevation of systemic L-KYN concentration, which is primarily thought to be neuroprotective in ischemia, but also linked to the pathophysiology of various neuropsychiatric diseases and systemic inflammatory response, may exert potential effects on the systemic vasoregulation or CBF in adult healthy animals.

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MATERIAL and METHODS

Animals and housing conditions

The experimental procedures were approved by the National Food Chain Safety and Animal Health Directorate of Csongrád County, Hungary. The procedures were performed according to the guidelines of the Scientific Committee of Animal Experimentation of the Hungarian Academy of Sciences (updated Law and Regulations on Animal Protection: 40/2013. (II. 14.) Gov. of Hungary), following the EU Directive 2010/63/EU on the protection of animals used for scientific purposes, and reported in compliance with the ARRIVE guidelines. Efforts were made to minimize the number of animals used and to reduce pain and discomfort. Before the experimental procedures, all the mice were in normal health and had no neurological deficits. All of the experiments were approved by the following ethical license: XX/01593/I/2010.

Surgical procedures

12-14 weeks old adult male C57Bl/6J mice (n=16, body weight 28±3 g) were used. The animals were obtained from The National Institute of Oncology (Budapest, Hungary), were group-housed under normal 12-h light/dark cycle, with *ad libitum* access to food and tap water. On the day of experiments, the animals were anesthetized with isoflurane (1.5% induction, 1% maintenance in N_2O:O_2, 70:30%) and allowed to breath spontaneously through a head mask. In order to avoid the production of airway mucus, atropin (0.1%, 0.01 ml) was injected intramuscularly shortly before the surgical procedures. Body temperature was maintained at 37˚C using a thermostatic heating pad (TMP-5b, Supertech Kft., Pécs, Hungary). The left femoral artery was cannulated for the continuous acquisition of mean arterial blood pressure (MABP). Then, the mice were transferred to a stereotaxic frame (David Kopf Instruments, CA, USA) and fixed in a prone position. A midline incision was made above the sagittal suture and the scalp was gently pulled aside. Lidocain (1%) was administered topically before opening the skin. The periosteum was removed with blunted forceps, the skull surface was rinsed with saline, and then covered with a thin layer of UV light adhesive (UV683 Light Curing Adhesive, Permabond Ltd., Wessex, UK) to prevent drying throughout the measurement. CBF was assessed through the intact parietal bone with the aid of laser speckle contrast imaging (PeriCam PSI HR System®, Perimed, Järfälla, Sweden) as described in detail elsewhere (Ayata and Dunn, 2004). Briefly, the field of view centered on the bregma was illuminated in a diffuse manner by a laser diode at 785 nm wavelength, and raw speckle images were captured by a camera positioned 10 cm above the

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animal's head. The field of view was adjusted to cover an area of 10 x 13 mm at high magnification of 20 μm/pixel covering both hemispheres. Raw speckle images were obtained at a sampling rate of 0.33 Hz, and computed to color-coded contrast images (Figure 1A) by a dedicated software (PimSoft 1.5.4.8, Perimed, Järfälla, Sweden). In summary, laser speckle flowmetry is a quantitative tool to assess noninvasively two-dimensional hemodynamic changes in the pial circulation of mice. Data obtained by this method informs about a relative perfusion level of the observed cortical area with high temporal and spatial resolution, whereas elevation in the values denote cerebral hyperemia, and decrease denote cerebral hypoperfusion.

Drug administration and experimental protocols

Animals were randomly assigned to two different treatment protocols (n=8/group). After taking a baseline of 20 min, a bolus injection of L-KYNs (300 mg/bwkg) or vehicle (0.1 M phosphate buffer) of the same volume (0.2 ml) was administered intraperitoneally. Subsequently, MABP and CBF were continuously monitored at least for 2.5 hours. All chemicals were purchased from Sigma, St. Louis, MO, USA.

Data analysis

All variables were simultaneously acquired and stored using a personal computer with dedicated softwares (PeriSoft 2.5.5 for BP and PimSoft 1.5.4.8 for CBF Perimed, Järfälla, Sweden). Data analysis was conducted by custom written scripts in a MATlab environment (MathWorks Inc.,MA, USA).

To determine the changes in CBF, identical regions of interest were positioned over each hemisphere, over the somatosensory cortical area (Figure 1A and C). The size of this area was 2.5±0.3 mm^2. Raw CBF changes were expressed relative to baseline by using the average CBF value of the first 5 min (100%) and the recorded biological zero obtained after terminating the experiment (0%) as reference points. CBF values were smoothed to the median of 5 points (15-second periods), and a moving average of 10 points (30-second periods) was calculated to decrease the inherent noise of the signal (Mahé et al., 2011). The integrity of cerebral autoregulation was determined by calculating a cerebrovascular autoregulatory index ($rCBF_x$) as described in detail elsewhere (Hinzman et al., 2014). First, MABP was downsampled to 0.33Hz. Second, Pearson correlation coefficients were calculated between 20 consecutive (300 seconds) time averaged data points (15-second periods) of MABP and CBF. Based on previous studies, $rCBF_x<0.3$ was accepted to indicate intact cerebrovascular autoregulation (Zweifel et al., 2008).

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In order to discriminate significant CBF variations reliably, a range of normal CBF variation was determined according to a threshold level (±1.5 stdev) for each individual measurement (Figure 1B and D). Because of spontaneous vasomotor activities and inherent noise of the signal, the CBF of the isoflurane-anesthetized mice is appreciably fluctuates. In order not to involve these to our analyses, we focused only to the slower components of the CBF variation. Consequently, when CBF dropped below the determined threshold range for more than 15 seconds (5 consecutive time points), a cerebral hypoperfusion transient, when exceeded that, a cerebral hyperemic transient was noted and the events were taken for further quantitative analysis. Time periods with $rCBF_x>0.3$ were excluded from the analysis in order to disregard passive CBF variations independent of drug effect. The following elements of the CBF transients were characterized: peak amplitude, duration, and magnitude expressed as area under the curve (AUC) (Fig.1D).

Statistical analysis was performed using the software SPSS (IBM SPSS Statistics for Windows, Version 22) or the inbuilt statistical functions of MATLAB (MathWorks Inc., MA, USA). The distribution of the data was tested with the Shapiro-Wilk normality test. Outliers were filtered with Grubbs test. For repeated measures with within-subjects factor of time and between-subjects factor of treatment, a mixed analysis of variance (ANOVA) model was applied on time averaged 30-min epochs of the MABP. Variables derived from the CBF variations were evaluated with Welch two sample t-test with Bonferroni correction to control type one error rate. Correlation analysis relied on a one-tailed Pearson test. Data are given as mean±stdev and levels of significance were defined as $p^{**}<0.01$, and $p^{***}<0.001$.

RESULTS

Systemic blood pressure

MABP was stable in both experimental groups throughout the entire data acquisition period. The mean MABP calculated for the total duration of data acquisition was 69.46±4.24 mmHg in the control group and 72.99±5.63 mmHg in the L-KYNs group. Statistical analysis revealed no significant difference neither between the defined epochs nor between the treatments.

Cerebral blood flow variation in the somatosensory cortex

The relative CBF values derived from the left and right hemispheres revealed strong, positive correlation ($r\geq0.95^{***}$), therefore they were averaged and analyzed as a single value for each animal.

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The frequency and the magnitude of hyperemic transients were negligible in both experimental groups. In the control group, hypoperfusion transients occurred in some but not all of the animals, and their temporal distribution was quite random. Conversely, hypoperfusion transients evolved in all the L-KYNs-treated animals, typically emerged 60-120 min following L-KYNs administration, and were markedly more pronounced (Fig. 2). As such, the peak amplitude of the hypoperfusion transients was greater in the treated group (-22.16±5.9%) compared to the control (-12.74±2.6%), $t(14.073)=5.175$, $p<0.001$ (Fig. 2A). The CBF reduction lasted longer in the treated group (6.5±3.2 min) than in the control (2.1±1.2 min), $t(11.545)=-4.201$, $p=0.003$ (Fig. 2B). In addition, the magnitude of the CBF reduction characterized by the AUC was greater in the treated group (5348±2350% x s) than that in the control (1199±580% x s), $t(9.692)=-5.48$, $p<0.001$ (Fig. 2C). Cerebral autoregulation remained intact during the hypoperfusion transients, as reflected by the rCBFx values under 0.3 (Fig.1C and D).

DISCUSSION

Here we have shown that the L-KYNs treatment destabilized resting CBF of isoflurane-anesthetized mice by inducing a number of transient hypoperfusion events, with no simultaneous influence on MABP. The present data are at variance with previous observations attributing a vasodilator and blood pressure lowering effect to L-KYN (Sas et al., 2003; Wang et al., 2010), which may be due to the various L-KYN concentrations used, or the induction or lack of anesthesia, or species difference. Isoflurane dose used in our study causes minimal cardiac depression (Janssen et al., 2004), but, along with nitrous oxide (DiSesa et al., 1987) it might mask the blood pressure attenuating effect of L-KYN. On the other hand, the blood pressure attenuating effect of L-KYN was observed during systemic inflammation or hypertension only (Kwok et al., 2002; Wang et al., 2010), and is very likely insignificant in naïve animals. It was previously demonstrated, that administration of L-KYN at low dose promoted hyperemic responses in awake rabbits (Sas et al., 2003). Conversely, the current data show that high-dose L-KYNs treatment induces transient CBF reductions in anesthetized mice. Kynurenine pathway metabolites are normally present in mammals. However, variations in inducibility, spatio-temporal expression pattern of the enzymes during KYN catabolism are not completely clarified. Leklem and co-workers found that, urinary excretion of metabolites following oral loading of tryptophan, L-kynurenine or 3-hydroxy- DL-kynurenine,was much greater in rats as compared to cats (Leklem et al., 1969). Age-

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dependent changes in the expression of kynurenine aminotransferase (KAT) isoformes in mice was also described (Yu et al., 2006). Furthermore, physiological concentration of KYNA reveals prominent differences among mammalian species (Moroni et al., 1988). In summary, kynurenergic manipulation may exert age and species-dependent effect.

Dose-dependent responses to various kynurenergic manipulations can be diverse. As such, KYNA was shown to facilitate excitatory postsynaptic potentials at low concentration, but it exerts inhibitory effect at higher-concentration, through dual actions on α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (Prescott et al., 2006; Rózsa et al., 2008). Therefore, it is reasonable to propose that the effect of L-KYNs on CBF is also dose dependent.

In the brain two spatially distributed routs exist for L-KYN metabolism: the quinolinic acid and the KYNA branches (Amori et al., 2009). The main source of quinolinic acid is the activated microglia, thus in a healthy, intact brain, catabolic activity of the enzymes located in this branch are relatively low (Guillemin et al., 2001). Therefore a bolus injection of L-KYNs is expected to be converted to KYNA with irreversible transamination catalyzed by kynurenine aminotransferases, located in perivascular astrocytes (Swartz et al., 1990) and in GAD67-immunopositive interneurons (Herédi et al., 2016). While our experimental conditions are likely to promote KYNA formation from L-KYNs, a modest increment of other downstream metabolites of the kynurenine pathway cannot be excluded. Indeed, their involvement in vasomotor regulation to the best of our knowledge has not been revealed. Since L-KYNs was administered in a buffered solution, furthermore, the brain KYNA production falls into the picomolar or nanomolar range, we propose that the *de novo* produced KYNA does not exert any effect on brain extracellular pH level.

Cerebral hypoperfusion transients occurred over the time when L-KYN is known to penetrate the brain tissue and *de novo* synthetized KYNA is released to the extracellular space (Swartz et al., 1990; Zmarowski et al., 2009). This brief time window (60-120 min) following L-KYNs administration is thought to be insufficient for the accumulation of other kynurenine pathway metabolites in the extracellular space. Consequently, the observed CBF dysregulation is likely to be related to an elevated concentration of brain extracellular KYNA.

While the molecular mechanism leading from elevated level of KYNA to various actions on different cell surface receptors to fluctuation in the extracellular level of distinct neurotransmitters to behavioral alterations are extensively investigated and increasingly elucidated (Schwarcz, 2016; Schwarcz et al., 2012; Vécsei et al., 2013), the mechanisms

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leading to vascular diameter changes remain to be clarified. Clearly, a number of possible processes could be operative in our model.

Cerebral cortical arteries were shown to express functional Mg^{2+}-insensitive NMDA receptors on their endothelial surface, whereas binding of KYNA was verified to inhibit glutamate induced vasodilation via an endothelial nitric oxide synthase (eNOS) dependent way (LeMaistre et al., 2012). However, the relevance of this pathway *in vivo* was not confirmed yet, and thought to be significant during enhanced glutamate release typical of ischemic brain injury or epilepsy. Nevertheless, inhibition of endothelial NMDA receptor owing to KYNA accumulation is very feasible (LeMaistre et al., 2012). Considerable amount of KYNA is present in the blood circulation due to peripheral metabolization of L-KYN. Thus, attenuation of eNOS-dependent vasodilation, which has a major role in the regulation of the resting CBF (Attwell et al., 2010), could be accounted for the observed dysregulation in the pial circulation.

The synthesis and release of vasodilator prostaglandins from astrocytes is driven by the fluctuation of intracellular Ca^{2+} concentration (Attwell et al., 2010; Howarth, 2014). Cortical astrocytes too express non-neuronal NMDA receptor (Lalo et al., 2006) and GPR35, a G_i-protein coupled receptor (Moroni et al., 2012). KYNA action on both receptor types leads to attenuated intracellular Ca^{2+} levels, associated with hindered release of vasoactive substance and impaired control of CBF. Furthermore, astrocytes contribute to the management of basal CBF by tonic regulation of blood supply via steady-state vasodilation of cortical arterioles. In acute rat brain slices of sensory-motor cortex, reduction in astrocyte Ca^{2+} concentration promoted vasoconstriction in adjacent arterioles, which can be mimicked in awake mice with the blockade of cyclooxygenase-1 enzyme (Rosenegger et al., 2015). Moreover, the inhibition of cyclooxygenase-1 enzyme, which is predominantly harbored in astrocytes, was shown to reduce resting CBF in mice (Niwa et al., 2001). Thus, this astrocytic pathway could be implicated in the cerebral hypoperfusion transients in response to high dose L-KYNs administration.

Interestingly, topical application of KYNA did not affect resting cerebellar CBF in anesthetized rats (Li and Iadecola, 1994), nor did it affect the diameter of pial arterial vessels in anesthetized newborn piglet (Bari et al., 2006). Still, when NMDA-receptor dependent vasodilation was induced either by NMDA or electrical stimulation, KYNA dose-dependently attenuated evoked postsynaptic currents and vascular dilatory responses (Bari et al., 2006; Li and Iadecola, 1994). However, it should be noted, that discrepancies may evolve due to anatomical (cerebellar vs cerebral) or age differences (newborn vs adult). Correspondingly,

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similar CBF reduction to what we observed was triggered in intact rat parietal cortex with MK-801, a non-competitive antagonist of NMDA receptor (Park et al., 1989). Accordingly, these findings may reinforce NMDA receptors as one of the target involved in KYNA-dependent hypoperfusion. The conceivable mechanisms via NMDA responses can be interpreted were discussed partly (non-neuronal NMDA receptors). However, the possibility that the KYNA-induced hypoperfusion are secondary to a decreased neuronal-network activity, and glucose utilization cannot be excluded (Birch et al., 1988; Busija et al., 2007; Schwarcz, 2016; Schwarcz et al., 2012; Vécsei et al., 2013). In support of this view, intracerebroventricular administration of KYNA induced mild suppression of electrocorticographic activity in anesthetized rats (Yokoi et al., 1998), and a net neuronal hyperpolarization, was found to be associated with concurrent pial arteriolar vasoconstriction in rat somatosensory cortex (Devor et al., 2007). Could the cerebral hypoperfusion transients found here reflect a lowered neuronal net metabolism? To answer this question further experiment should be done focusing on the glucose utilization and the electrophysiological activity of the cerebral cortex.

The observed KYNA-related cerebral hypoperfusion transients occurred almost simultaneously in both hemispheres in a similar manner, implying a global action of KYNA. On the other hand, we monitored CBF variations only in the exposed parietal cortical surface. Cholinergic inputs to the parietal cortex, ascend from subcortical neurons, located in the nucleus basalis of Meynert (Hamel, 2006; Sato et al., 2002). These afferents innervate cortical arteries, local nitric oxide synthase (NOS) and neuropeptide Y (NPY) immunoreactive interneurons and perivascular astrocytes. The tonic activation of cholinergic inputs promotes vasodilation in the cortex, mediated by nicotinic cholinergic receptors, while the selective inactivation of cholinergic neurons in the basal forebrain induce significant (24-40%) hypoperfusion, mainly in the posterior parietal and temporal cortical regions (Waite et al., 1999). It is possible that KYNA can significantly hinder the impact of basal forebrain cortical - cholinergic inputs, by attenuating acetylcholine release from the cholinergic nerve terminals and blocking α7nACh receptors located on interneurons (Zmarowski et al., 2009). Taken together, the increased concentration of KYNA may cause cerebral hypoperfusion transients in the parietal cortex by inhibition of its cholinergic innervation.

CONCLUSIONS

In summary, our data demonstrate that systemic administration of high-dose L-KYNs induces cerebral hypoperfusion transients while it does not alter systemic blood pressure. The

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impairment of the kynurenine pathway metabolism is increasingly considered to be involved in the occurrence and the progression of metabolic dysfunctions observed in various neurodegenerative diseases. Thus, manipulation of the availability of kynurenine metabolites as a potential therapeutic approach has been extensively investigated recently. Although, up to this point the possibility that this manipulation might interfere with the cerebrovascular regulation was not taken into account. In conclusion, the L-KYNs treatment destabilizes resting CBF by inducing a number of transient hypoperfusion events, a phenomenon, that should be considered by interpreting the effect of kynurenergic manipulation on brain function.

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FIGURE CAPTIONS

Fig. 1. Representative cerebral blood flow (CBF) images of the cortical surface were obtained with laser speckle contrast analysis, and subsequently pseudo-colored to visualize the intensity of perfusion in a vehicle-treated animal **(A)** and an L-KYNs-treated mouse **(B)**. Regions of interest used for CBF assessment are outlined with black in the images. Representative traces in panels C and D show variation in CBF (red trace), mean arterial blood pressure (MABP; green trace), and cerebrovascular autoregulatory indexes (rCBFx, blue trace) taken from a vehicle-treated **(C)** or an L-KYNs-treated **(D)** animal. A CBF threshold (±1.5 stdev of the mean; red horizontal dotted lines) was determined to evaluate CBF variations. CBF below this range was taken relevant for the quantitative analysis: peak amplitude (vertical arrow), duration (horizontal arrow), and area under the curve (AUC; grey colored area). The traces depict CBF variation without concomitant changes in MABP following vehicle or L-KYNs administration (vertical dotted line). Cerebral autoregulation was intact (rCBFx<0.3; red horizontal line) at times when CBF decreased below threshold (indicated by arrowheads). Hashtags above the CBF traces mark the time points when the speckle images in panel A and B were taken.

Fig. 2. Quantitative assessment of hypoperfusion transients after L-KYNs with respect to vehicle treatment: **(A)** Maximum amplitude of hypoperfusion transients; **(B)** Duration of hypoperfusion transients; **(C)** Magnitude of hypoperfusion transients, expressed as area under

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the curve. Data are given as mean±stdev. Statistical analysis relied on Welch two sample t-test with Bonferroni correction. Note the significant effect of the L-KYNs treatment on all of the observed parameters. Statistical significance was determined as $p^{**}<0.01$, and $p^{***}<0.001$.

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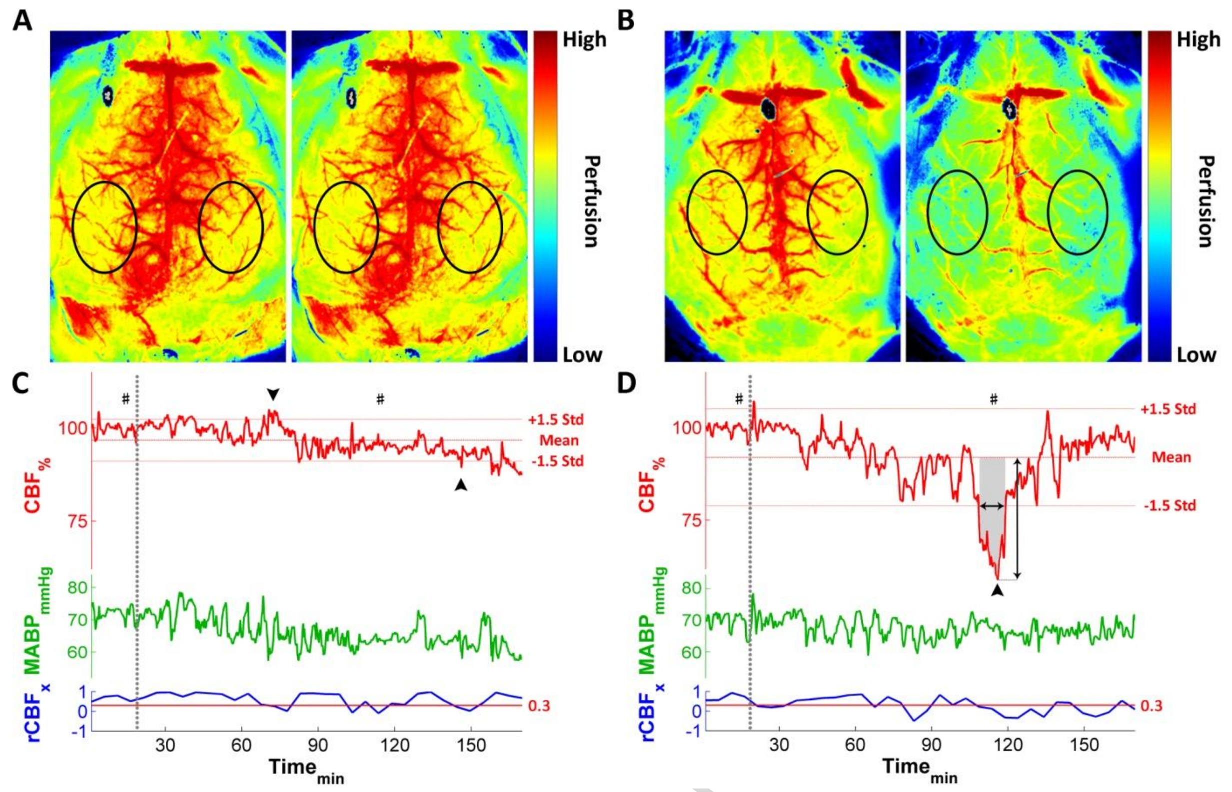

Fig. 1

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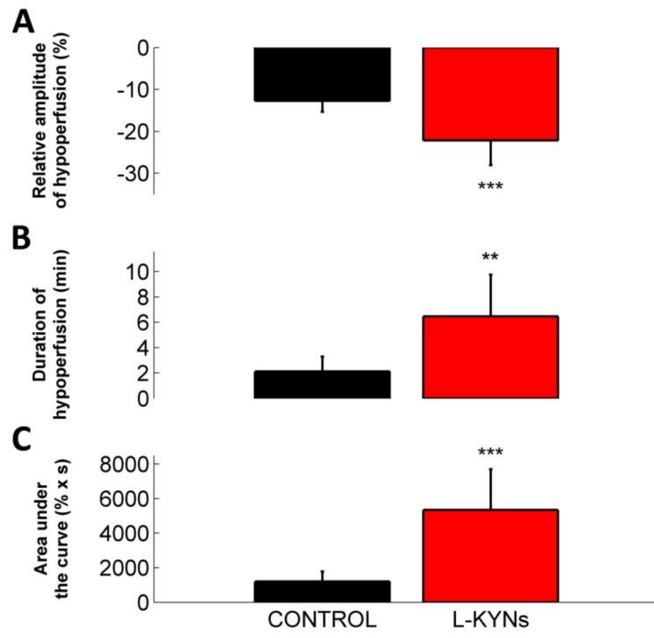

Fig. 2

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