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Effekt von akuter Hypoxie auf die Perfusion und Verfügbarkeit des A1-Adenosinrezeptors im menschlichen Gehirn

(AdenOx)

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Die dieser Arbeit zugrunde liegenden Experimente sind von mir mit Unterstützung der oben genannten Co-Autoren durchgeführt worden. Zudem wurden wir unterstützt von den medizinische Technologinnen für Radiologie Kerstin Kempter und Annette von Waechter und dem Techniker Manfred Schultze sowie Physiker Martin Wittkowski.

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Größter Dank gilt allen Probandinnen und Probanden, die sich für AdenOx zur Verfügung gestellt haben.

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Abkürzungsverzeichnis

[^{18}F]CPFPX:	8-Cyclopentyl-3-(3-[18F]Fluorpropyl)-1-propylxanthin
A_1AR:	A1-Adenosinrezeptor
AAT:	Arterial Arrival Time
AR:	Adenosinrezeptor
ASL:	Arterial Spin Labeling
EKG:	Elektrokardiogramm
F_iO_2:	inspiratorische Sauerstofffraktion
MRT:	Magnetresonanztomographie
PET:	Positronen-Emissions-Tomographie
PLD:	Post-Label Delay
PVT:	Psychomotor Vigilance Task
SpO_2:	periphere Sauerstoffsättigung
V_T:	Verteilungsvolumen

1. Zusammenfassung

Einleitung: In tierexperimentellen Studien wurde beobachtet, dass der inhibitorische Neuromodulator Adenosin während Hypoxie in den interstitiellen Raum des Gehirns freigesetzt wird. Die Aktivierung des A1-Adenosinrezeptors (A_1AR) durch Adenosin schützt das Gehirn vor Sauerstoffmangel und Überlastung, indem zerebraler Blutfluss, Stoffwechsel und elektrische Aktivität angepasst werden.

Methoden: Mithilfe von 8-Cyclopentyl-3-(3-[^{18}F]Fluorpropyl)-1-propylxanthin ([^{18}F]CPFPX), einem selektiven PET-Tracer für den A_1AR, wurde die Hypothese getestet, dass eine durch Hypoxie induzierte Adenosinfreisetzung die Verfügbarkeit von A_1AR im menschlichen Gehirn reduziert. Zusätzlich wurde untersucht, ob diese Reaktion mit Veränderungen der zerebralen Perfusion und der psychomotorischen Wachsamkeit assoziiert ist. Zehn gesunde Probandinnen und Probanden nahmen an einem 110-minütigen PET/MRT-Hybridexperiment teil, wovon 30 Minuten bei normobarer Hypoxie mit einer peripheren Sauerstoffsättigung (SpO_2) zwischen 70 % und 75 % absolviert wurden. Die Perfusion des Gehirns wurde mittels Arterial Spin Labeling (ASL) in hoher zeitlicher Auflösung gemessen. Alle 10 Minuten wurde ein 3-minütiger Reaktionstest zur psychomotorischen Wachsamkeit durchgeführt. Herzfrequenz, Atemfrequenz und periphere Sauerstoffsättigung wurden kontinuierlich überwacht.

Ergebnisse: Akute normobare Hypoxie führte in allen sieben untersuchten Gehirnregionen zu einer signifikanten Reduktion der A_1AR-Verfügbarkeit (z.B. Frontallappen: 13,5 %; P = 0,0144), während die Perfusion der grauen Substanz signifikant anstieg (z.B. Frontallappen: 42,5 %; P = 0,0007). Die Herzfrequenz erhöhte sich signifikant um 19 % (P = 0,0039) und die durchschnittliche Reaktionsgeschwindigkeit verringerte sich signifikant um 4,3 % (P = 0,0021).

Schlussfolgerung: Nach unserem Wissen zeigt diese Studie erstmals beim Menschen, dass akute Hypoxie, entsprechend einem Sauerstoffpartialdruck auf einer mittleren Höhe von etwa 5.500 m, die A_1AR-Verfügbarkeit im Gehirn reduziert. Dieses Ergebnis unterstützt die

Hypothese, dass durch Hypoxie induzierte Adenosinfreisetzung zu einer erhöhten Belegung des A_1AR führt.

2. Einleitung

Adenosin sammelt sich tagsüber als Produkt des Neuronenstoffwechsels im Gehirn an und erzeugt so über das Andocken an Adenosinrezeptoren (AR) Müdigkeit und Schläfrigkeit.[1] Adenosin entsteht dabei durch den Abbau von Adenosintriphosphat (ATP) und reguliert neuronale Aktivität und Homöostase.[2] Neben dieser wichtigen Funktion in der Schlaf-Wach-Regulation, spielt Adenosin auch in der neuronalen Hemmung und der damit verbundenen neuroprotektiven Wirkung unter pathophysiologischen Bedingungen wie Hypoxie oder Ischämie eine wichtige Rolle.[3]

Von den vier Subtypen (A_1, A_{2A}, A_{2B}, A_3) ist der A1-Adenosinrezeptor (A_1AR) im gesamten Gehirn am weitesten verbreitet.[4] Studien an Ratten deuten darauf hin, dass die Wirkung von Adenosin auf den A_1AR das Gehirn durch Anpassungen des zerebralen Blutflusses, des Stoffwechsels und der elektrischen Aktivität vor Sauerstoffmangel und Überanstrengung schützt.[5,6] Diese Tierstudien zeigten, dass ein Atemgas mit einem Sauerstoffgehalt von 8 bis 9% – entsprechend einer Höhe von 6000 bis 7000 m – je nach Bestimmungsmethode zu einem 2- bis 4-fachen Anstieg des Adenosinspiegels im Gehirn führt. Unter ischämischen Bedingungen werden so adaptive Veränderungen induziert, die durch o.g. Veränderungen das Gehirn schützen.[7] Adenosin als ein hemmender Neuromodulator wird während Hypoxie im Zwischenzellraum produziert oder freigesetzt. Die Adenosin-abhängige Aktivierung von A_1AR ist laut Studien an Nagern während einer zerebralen Ischämie von Vorteil, da die Aktivierung eine schnelle und umfassende Abnahme der synaptischen Aktivität bewirkt, wenn Mäuse Hypoxie oder Sauerstoff-Glukoseentzug ausgesetzt werden.[7]

2.1. Fragestellungen und Ziel der Arbeit

In dieser Studie untersuchten wir, ob ein 30-minütiges Intervall akuter Hypoxie mit einer peripheren Sauerstoffsättigung (SpO_2) zwischen 70 % und 75 % (Figure 1 und 4), die einem Sauerstoffpartialdruck der Atemluft in einer mittleren Höhe von ca. 5500 m entspricht, Auswirkungen auf die A_1AR-Verfügbarkeit im menschlichen Gehirn hat, die mit den o.g. Beobachtungen erhöhter Adenosinspiegel bei Nagetieren vergleichbar sind. Dieser Anstieg des endogenen Adenosins würde den radioaktiven Tracer verdrängen und somit die mittels

Positronen-Emissions-Tomographie (PET) gemessenen Tracerkonzentrationen verringern.[8] Darüber hinaus untersuchten wir andere Prozesse, die an der Vermittlung dieser Effekte beteiligt sind: Die Perfusion des Gehirns wurde mit der in der Magnetresonanztomographie (MRT) angewendeten Technik Arterial Spin Labeling (ASL) gemessen. Auch Vitalparameter wie die Herzfrequenz wurden bestimmt, um in Hypoxie bereits bekannte physiologische Regelkreise zu bestätigen.[9] Mit dem Psychomotor Vigilance Task (PVT) wurden die Auswirkungen auf Aufmerksamkeit und Reaktionszeit der Probandinnen und Probanden während des Sauerstoffmangels erfasst.[10]

Haupthypothese:

- Exposition gegenüber einem akuten Intervall von Hypoxie steigert die Konzentration von endogenem Adenosin im menschlichen Gehirn und reduziert so die Verfügbarkeit von A_1AR im Vergleich zur Normoxie.

Nebenhypothesen:

- Perfusion des Gehirns steigt in Hypoxie im Vergleich zu Normoxie in Abhängigkeit von der A_1AR-Verfügbarkeit an
- psychomotorische Wachsamkeit verringert sich in Hypoxie im Vergleich zu Normoxie in Abhängigkeit von der A_1AR-Verfügbarkeit

Diese Studie hat zum Ziel, die Auswirkungen von Hypoxie auf das zerebrale adenosinerge System, die Perfusion des Gehirns und die Aufmerksamkeit besser zu verstehen. Hierdurch soll das Wissen über den Metabolismus, die Verteilung und die Kinetik von Adenosin erweitert werden. Als einer der am weitesten verbreiteten Neuromodulatoren im menschlichen Gehirn spielt Adenosin eine entscheidende Rolle bei der Regulierung des Energiestoffwechsels und der neuronalen Aktivität, insbesondere unter Stressbedingungen wie Sauerstoffmangel. Erkenntnisse über o.g. Zusammenhänge könnten zur Entwicklung innovativer therapeutischer Strategien beitragen, die auf zerebrale Ischämie und andere mit hypoxischer zerebraler Dysfunktion zusammenhängende Erkrankungen abzielen.

2.2. Anteil Manuel Michno

Bereich	Tätigkeit	Anteil MM
Vorstudie	Planung und Durchführung einer Vorstudie zur Hypoxie-induzierten Veränderung von Hirnperfusion ohne Tracer zur Absicherung der Machbarkeit und Relevanz	Federführend
Versuchsplanung	Konzeption der Forschungsfrage, Entwicklung des Studiendesigns, Planung der Hypoxie-Exposition	Wesentliche Mitwirkung
Ethik und Genehmigungen	Vorbereitung der Unterlagen für Ethikantrag und Strahlenschutzgenehmigung	Federführend
Protokollerstellung	Detailplanung der Abläufe (z. B. PET/MRT-Protokoll, PVT-Zeitpunkte, Blutentnahme-Zeitfenster)	Federführend
Durchführung der Studie	Organisation und Koordination der Probandensitzungen, Aufsicht über PET/MRT, ASL und Hypoxiephasen	Übernahme sämtlicher Aspekte
Blutentnahmen/ Sicherheit	Abstimmung medizinischer Sicherheitsaspekte bei Hypoxie, Überwachung der Vitalparameter	Übernahme sämtlicher Aspekte
Bilddatenanalyse	Auswertung der PET- und ASL-Daten mittels PMOD, inklusive Berechnung der Verteilungsvolumina	Eigenständig durchgeführt
Statistische Auswertung	Durchführung von Mixed-ANOVAs, Wilcoxon-Tests, Normalverteilungsprüfung, Bonferroni-Holm-Korrekturen	Eigenständig durchgeführt
Vergleichskohorten-analyse	Aufbereitung und Abgleich mit historischen Normoxie-Daten von Pierling et al. (externer Vergleich)	Selbst initiiert & eigenständig durchgeführt
Manuskripterstellung	Verfassen des Manuskripts inklusive Abstract, Methoden, Ergebnisse, Diskussion und Supplement für JNM	Hauptverfasser

Effect of Acute Hypoxia Exposure on the Availability of A_1 Adenosine Receptors and Perfusion in the Human Brain

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In animal studies it has been observed that the inhibitory neuromodulator adenosine is released into the cerebral interstitial space during hypoxic challenges. Adenosine's actions on the A_1 adenosine receptor (A_1AR) protect the brain from oxygen deprivation and overexertion through adjustments in cerebral blood flow, metabolism, and electric activity. **Methods:** Using 8-cyclopentyl-3-(3-[^{18}F]fluoropropyl)-1-propylxanthine ([^{18}F]CPFPX), a PET tracer for the A_1AR, we tested the hypothesis that hypoxia-induced adenosine release reduces A_1AR availability in the human brain. Furthermore, we investigated whether this response is associated with altered brain perfusion and psychomotor vigilance. Ten healthy volunteers completed a 110-min bolus–plus–constant-infusion [^{18}F]CPFPX PET/MRI hybrid experiment including a 30-min interval of normobaric hypoxia with peripheral oxygen saturation between 70% and 75%. We obtained blood samples to calculate metabolite-corrected steady-state A_1AR distribution volumes and measured gray matter brain perfusion via arterial spin labeling in high temporal resolution. A 3-min psychomotor vigilance test was conducted every 10 min, and heart rate and peripheral blood oxygen saturation were continuously measured. **Results:** In all 7 examined brain regions, hypoxia reduced A_1AR availability significantly (e.g., frontal lobe, 13.5%; $P = 0.0144$) whereas gray matter brain perfusion increased (e.g., frontal lobe, 42.5%; $P = 0.0007$). Heart rate increased by 19% ($P = 0.0039$). Mean reaction speed decreased by 4.3% ($P = 0.0021$). **Conclusion:** Our study is the first, to our knowledge, to demonstrate that acute hypoxia, corresponding to a mean altitude of 5,500 m (18,000 ft), reduces A_1AR availability in the human brain. The finding is consistent with hypoxia-induced cerebral adenosine release leading to increased A_1AR occupancy.

Key Words: hypoxia; adenosine receptors; A_1AR; [^{18}F]CPFPX; PET; ASL

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Adenosine accumulates in the brain during the day and thus mediates fatigue and sleepiness via docking to adenosine receptors. Among the 4 types of adenosine receptors, the A_1 adenosine receptor (A_1AR) exhibits the most widespread distribution throughout the brain (*1*). Animal studies suggest that adenosine's actions on A_1AR protect the brain from oxygen deprivation and overexertion through adjustments in cerebral blood flow, metabolism, and electric activity (*2,3*). These studies on rats showed that a breathing gas with 8%–9% oxygen content corresponding to an altitude of 6,000–7,000 m led to 2- to 4-fold increases in adenosine in the cerebral cortex depending on the method used for determination. Under ischemic conditions, adaptive changes are induced, such as changes in cerebral blood flow, metabolism, and electric activity (*4*). Adenosine, as an inhibitory neuromodulator, is produced in or released to the interstitial space during hypoxia and thought to mediate several of these effects. Adenosine-dependent activation of A_1AR is beneficial during cerebral ischemia because its activation causes a rapid and profound decrease in synaptic activity when mice are exposed to hypoxia or oxygen-glucose deprivation (*4*).

In this study, we investigated whether a 30-min interval of acute hypoxia with peripheral oxygen saturation (SpO_2) of between 70% and 75% (Fig. 1), corresponding to oxygen saturation at a mean altitude of approximately 5,500 m (18,000 ft), has effects on A_1AR availability in human brains comparable to observations of increased adenosine levels in rodents. This hypothetical increase in endogenous adenosine would displace the tracer and thus reduce the measured levels of the tracer (*5*). In addition, we investigated other systems involved in the mediation of these effects. Gray matter brain perfusion was measured using arterial spin labeling (ASL). Vital parameters such as heart rate were also determined to confirm known physiologic hypoxic control circuits (*6*). A psychomotor vigilance test (PVT) measured the effects on sustained attention in subjects during oxygen deprivation.

MATERIALS AND METHODS

Study Protocol

All experimental procedures were approved by the ethics committee of the Medical Association of North Rhine, Germany, and the German

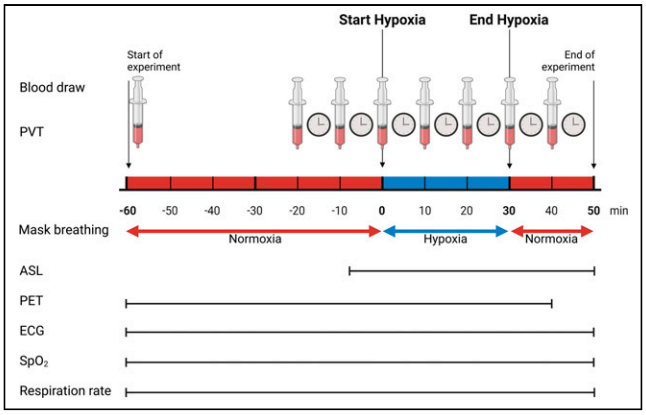

FIGURE 1. PET/MRI 110-min protocol: blood sampling (syringe symbols) at minutes −58, −20, −10, 0, 10, 20, 30, and 40; PVT (clock symbols, 3-min duration) at minutes −15, −5, 5, 15, 25, 35, and 45; normoxia with pressured air (21% O_2) from −60 to 0 min and from 30 to 50 min; hypoxia with mixture of pressured air (21% O_2) and nitrogen-enriched pressured air (8% O_2) from 0 to 30 min; and ASL sequence from −8:30 to 50 min; PET acquisition from −60 to 40 min. Electrocardiography, SpO_2, and respiration rate were monitored or acquired continuously. ECG = electrocardiography.

Federal Office for Radiation Protection. The procedures were conducted according to the principles of the Declaration of Helsinki. All participants gave written informed consent.

Ten healthy, right-handed human volunteers (3 female and 7 male; mean age ± SD, 31.3 ± 8.9 y [range, 19–46 y]) were included in the study. Body mass index was 24.1 ± 2.7 kg/m^2, and injected activity was 208.3 ± 39.1 MBq, which equals 2.70 ± 0.16 MBq of activity per kilogram of body weight (supplemental materials, available at http://jnm.snmjournals.org) (*7–45*).

Breathing-Gas Mixture

A quickly adjustable mixture of the breathing gas was achieved by combining pressurized air (21% oxygen concentration) and nitrogen-enriched pressured air (8% oxygen concentration, 13% higher nitrogen concentration than in normal air) (supplemental materials).

PET/MRI Data Acquisition

PET and ASL data, supplemented by anatomic high-resolution 3-dimensional T1-weighted MR images, were acquired using a 3-T PET/MRI system (Biograph mMR [VE11P]; Siemens Healthineers) (*46*). The PET/MRI system was equipped with a 32-channel head coil.

ASL

Gray matter brain perfusion was measured using a 3-dimensional gradient spin-echo sequence covering the whole cerebrum with pseudocontinuous ASL and background suppression, using CAIPIRINHA (Controlled Aliasing in Parallel Imaging Results in Higher Acceleration; Siemens) k-space sampling developed by the German Center for Neurodegenerative Diseases (*47*). Multiple postlabel delays of 0.25, 0.80, 1.35, and 1.90 s effectively covered the expected shift in arterial arrival time during hypoxia (*48,49*). Correspondingly, the referred mean gray matter arterial arrival time in all subjects was 1.04 s in normoxia and 0.94 s in hypoxia, which were well within the range of postlabel delays and equal a shift of 10% (supplemental materials).

[^{18}F]CPFPX PET Data Acquisition and Processing

A_1AR availability was quantified using PET with the highly selective and well-established tracer 8-cyclopentyl-3-(3-[^{18}F]fluoropropyl)-1-propylxanthine ([^{18}F]CPFPX), following the methodology described in previous studies (*50–52*). This method is based on competitive binding of [^{18}F]CPFPX and the endogenous agonist adenosine to A_1AR in the human brain (*5*).

[^{18}F]CPFPX was formulated and synthesized as previously described (*53*). Chemical purity was always greater than 96%. The radioligand was diluted with sterile saline (0.9%) and administered using a standard syringe pump. The radiotracer was injected as an intravenous bolus (15.9 mL in 2 min) followed by a constant infusion (34.1 mL in 118 min) with a K_{bol} value (total time required to infuse the bolus volume at the infusion rate) of 55 min (*54*). This bolus plus constant infusion led to equilibrium of the tracer throughout the experiment and especially during hypoxia (Supplemental Fig. 1). Injection and scan were started simultaneously (supplemental materials).

PET and corresponding MRI data were preprocessed using PMOD Neuro Tool (version 4.1; PMOD Technologies), following the methodology previously described (supplemental materials) (*55*).

Kinetic modeling was performed using PMOD Kinetics Tool. Regional time–activity curves were calculated for each voxel of interest. A decay-corrected whole-blood function and a decay-, metabolism-, and extraction-corrected plasma input function were used to correct the regional time–activity curves. The corrected time–activity curves were used to estimate metabolite-corrected steady-state A_1AR distribution volume (V_T). V_T at equilibrium (between 40 and 100 min) corresponds to the ratio of the radioligand concentration in the target region in the tissue (kBq × cm^{-3}) to the plasma activity (kBq ×mL^{-1}) (*56*).

Vital Parameters

We determined heart rate using 2 measurement methods at 3 locations. Two independent SpO_2 sensors were used to ensure safety and collect reliable data (supplemental materials).

PVT

The PVT is a visual response test that shows a minor learning curve (*57*). It is widely used in studies related to human sleep and chronobiology to assess sustained attention. Since the 3-min PVT is as reliable as the 10-min PVT (*58*), this shortened version was used so as not to interact with blood sampling (supplemental materials).

Statistical Analyses

Excel (Microsoft), MATLAB (MathWorks), and SAS (version 9.4; SAS Institute) software were used for statistical testing. Values were reported as mean and SD unless stated otherwise.

Because of various compensation mechanisms (*7*), we used 20 min (PET), 7 min (ASL), 8 min (heart rate), and 2 PVTs before the onset of hypoxia to determine the mean normoxic values and only the second half of hypoxia (minutes 15–30 after the onset of hypoxia) to determine the mean values for hypoxia.

Mixed ANOVAs with subject as a random factor and condition (normoxia, hypoxia), region (7 brain regions), and interaction between condition and region as fixed factors were used for analysis of PET and ASL data. Results were adjusted post hoc for multiple comparisons with the Bonferroni–Holm procedure. PVT speed was analyzed with a random-subject mixed ANOVA including condition as a fixed factor. Normal distribution of residuals was confirmed with Q–Q plots and Shapiro–Wilk tests. Normal distribution of residuals could not be achieved for heart rate. A Wilcoxon signed-rank test was used to compare heart rate between conditions. The significance level was 5%.

Comparison to Control Group from Previous Study

In Figure 2A, our subjects' A_1AR availability over time is plotted together with A_1AR availability taken from Pierling et al. (*55*). Subjects were matched for age, sex, and body mass index, and the data were collected in the same scanner with the same scanning protocol. We used the data from Pierling et al. only for illustrative purposes to show the time course of our data compared with a normoxic control group.

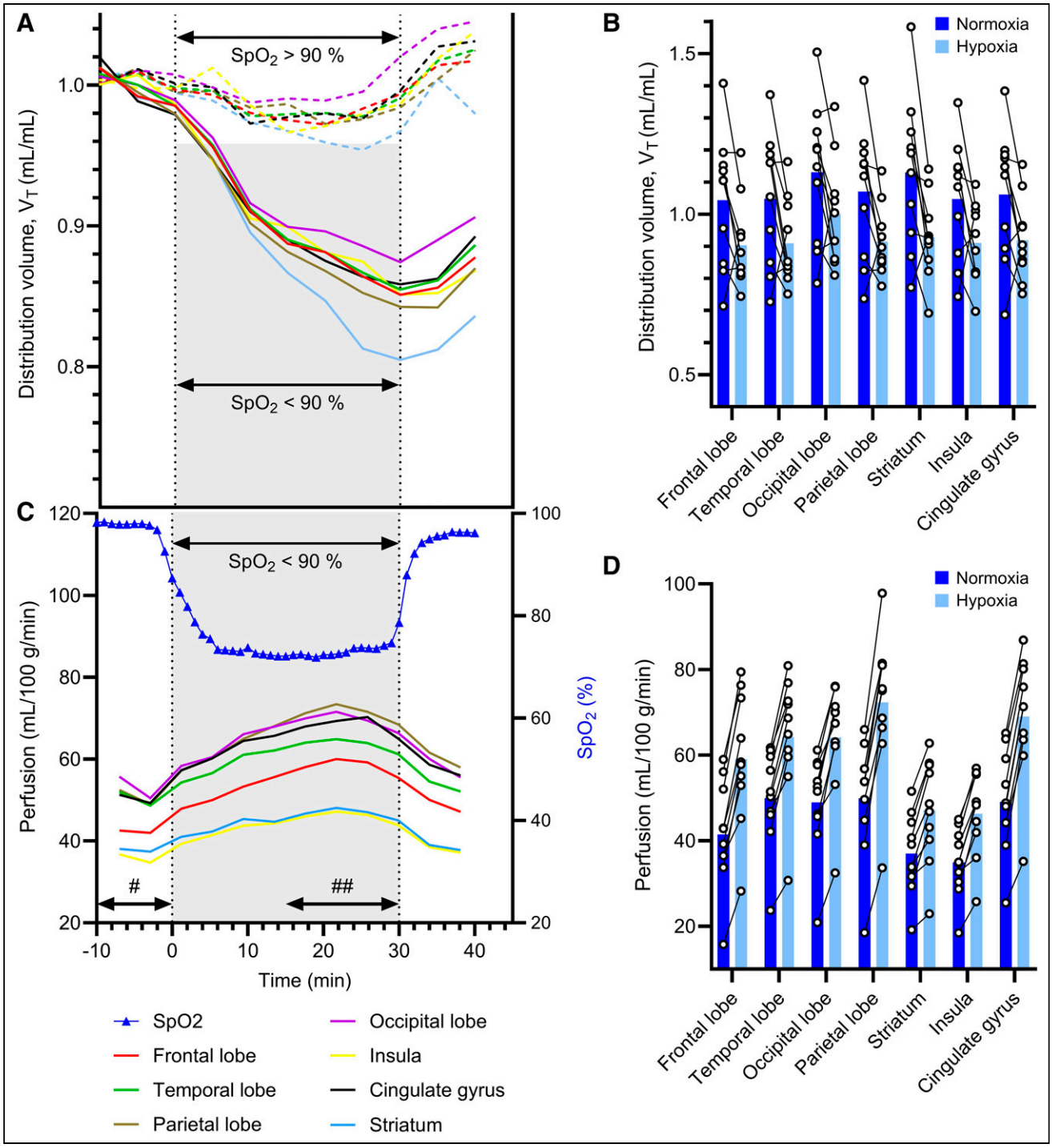

FIGURE 2. V_T and brain perfusion under normoxia and hypoxia. (A) A_1AR availability (V_T) decreased in hypoxia (solid lines) compared with control group measured in normoxia for visualization purposes (dotted lines). Data were normalized to first 4 data points (10 min). Normalization was done by dividing each data point by mean of first 4 data points for each of 2 groups. Control group (dotted lines) measured with same PET protocol in same scanner showed no change in A_1AR availability, whereas subjects of present study (solid lines) showed significant decrease in A_1AR availability in all 7 brain regions. (B) A_1AR availability decreased in hypoxia: columns show mean V_T values, and connected points show individual values. (C) Gray matter cerebral perfusion increased in examined brain regions in hypoxia compared with normoxia. Each time point is averaged out of 3 perfusion values, each being generated from 4 postlabel delays; 0 min is start of hypoxia. For statistical analysis, normoxia was defined as time before 0 min (#) and hypoxia was defined as 15–30 min of hypoxia (##). (D) Perfusion increased in each brain region under consideration: columns show mean values, and connected points show individual values of each subject. Shaded area labels $SpO_2 < 90\%$, and switch to hypoxia occurred about 5 min earlier.

RESULTS

A_1AR Availability Decreased During Hypoxia

Mixed ANOVA indicated significant differences between conditions ($P < 0.0001$) but not between regions ($P = 0.0588$) or for the interaction ($P = 0.9505$). During hypoxia, metabolite-corrected steady-state A_1AR V_T, which reflects A_1AR availability as determined by PET, was significantly reduced in all 7 examined brain regions of the automated anatomic labeling–merged atlas (Table 1; Figs. 2A, 2B, 3A, and 3B). The reductions in V_T ranged from an 11.5% decrease in the occipital lobe to a 17.9% decrease in the striatum. Additionally, changes in occupancy levels from Lassen plots were 10.3% ± 19.7%.

The reduction in the availability of A_1AR becomes evident especially in direct comparison with the normoxia control group (Fig. 2A).

Brain Perfusion Increased During Hypoxia

Mixed ANOVA of ASL data showed highly significant results (condition, region, and interaction: all $P < 0.0001$). Mean gray matter brain perfusion of all evaluated brain regions significantly increased between 27.4% and 44.9% (Table 2; Figs. 2C, 2D, 3C, and 3D). An increase in perfusion occurred almost instantaneously after the onset of hypoxia and plateaued about 20 min after the onset of hypoxia (Fig. 2C).

Heart Rate Increased During Hypoxia

Heart rate during hypoxia increased on average by 19% compared with normoxia ($P = 0.0039$). The time course is shown in Figure 4. Heart rate increased markedly already before the SpO_2 dropped below 90%. The heart rate plateaued after approximately 5 min.

Reaction Time on the PVT Was Longer During Hypoxia

The average reaction time of the subjects was prolonged by 4.6% or 12 ms during hypoxia (Fig. 5), and correspondingly, reaction speed was significantly reduced by 4.3% ($P = 0.0021$). The reduction in PVT speed was stable after 15 min of hypoxia.

Correlation Was Not Significant Between Relative Change in V_T, Perfusion, and Psychomotor Vigilance

No significant linear or monotonic correlation (Pearson and Spearman) between the relative changes in V_T, perfusion (respectively for each brain region defined in the automated anatomic labeling–merged atlas), or psychomotor vigilance was found (Supplemental Fig. 2; Supplemental Tables 1 and 2).

TABLE 1
A_1AR Availability in 7 Brain Regions: Hypoxia vs. Normoxia

Brain region	Normoxia (mL/mL)	Hypoxia (mL/mL)	Relative change (%)	*P*
Frontal lobe	1.04 ± 0.07	0.90 ± 0.04	13.5	0.0144
Temporal lobe	1.05 ± 0.07	0.91 ± 0.04	13.2	0.0144
Occipital lobe	1.13 ± 0.07	1.00 ± 0.05	11.5	0.0144
Parietal lobe	1.07 ± 0.07	0.91 ± 0.03	14.6	0.0063
Striatum	1.13 ± 0.08	0.93 ± 0.04	17.9	0.0008
Insula	1.05 ± 0.06	0.91 ± 0.04	13.1	0.0144
Cingulate gyrus	1.06 ± 0.07	0.92 ± 0.04	13.4	0.0144

Data mean ± SEM; *P* values were Bonferroni-Holm–adjusted.

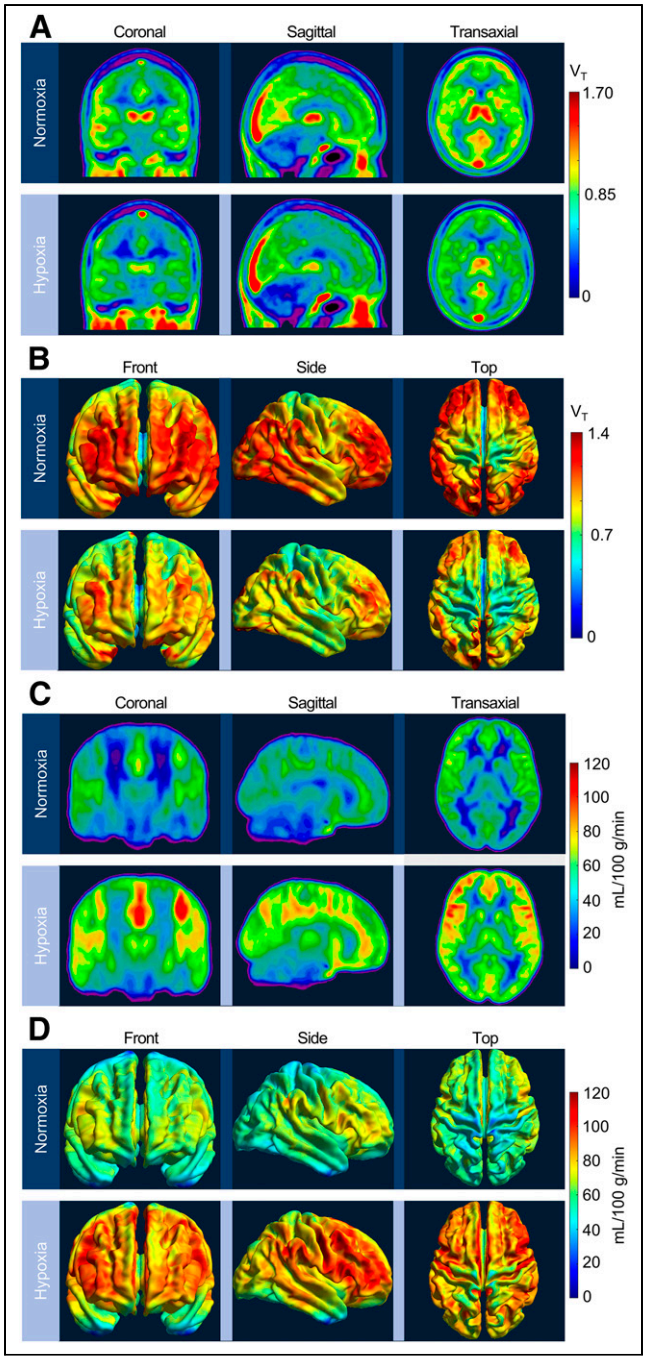

FIGURE 3. Color-coded V_T and brain perfusion under normoxia and hypoxia. (A and B) Average images of [^{18}F]CPFPX V_T show higher A_1AR availability in normoxia than in hypoxia: planar parametric images (A) and surface representations (B). (C and D) Average images of gray matter brain perfusion measured with ASL show increase in perfusion during hypoxia: planar parametric images (C) and surface representations (D).

DISCUSSION

A_1AR Availability

We examined the effects of a 30-min acute hypoxia exposure leading to an SpO_2 of just above 70% on the well-rested human brain in

TABLE 2
Brain Perfusion in Different Brain Regions: Hypoxia vs. Normoxia

Brain region	Normoxia (mL/100 g/min)	Hypoxia (mL/100 g/min)	Relative change (%)	*P*
Frontal lobe	41.5 ± 4.0	59.1 ± 4.9	42.5	0.0007
Temporal lobe	50.0 ± 3.6	64.3 ± 4.5	28.6	0.0007
Occipital lobe	48.9 ± 3.7	64.2 ± 4.1	31.1	0.0007
Parietal lobe	49.9 ± 4.3	72.3 ± 5.3	44.9	0.0007
Striatum	37.0 ± 3.7	47.1 ± 4.5	27.4	0.0007
Insula	34.9 ± 2.5	46.3 ± 3.1	32.6	0.0007
Cingulate gyrus	49.1 ± 4.2	69.0 ± 5.2	40.7	0.0007

Data mean ± SEM; *P* values were Bonferroni-Holm–adjusted.

10 healthy subjects. Our main finding was a globally reduced cerebral A_1AR availability ranging from 11.5% in the occipital lobe to 17.9% in the striatum. The findings could also be reproduced in the Lassen plot, showing the change in occupancy of the A_1AR. These differences were observed in all brain regions investigated in a pattern pointing to a rise of endogenous adenosine in hypoxic conditions. Moreover, these results present the first indirect evidence of rising adenosine levels during hypoxia in humans. Respective hypotheses established on the basis of animal models (*2*–*4*) were thus confirmed.

Compared with mice, we expected a milder increase in humans, as the mice received less oxygen (8%–9% vs. 10%) and underwent several cycles of hypoxia. Other environmental conditions, such as halothane anesthesia, also affect comparability. The more extreme environment the mice were exposed to resulted in a drop in blood pressure from 112 to 70 mm Hg, a drop in partial pressure of CO_2 from 39 to 25 mm Hg, and an increase in pH from 7.39 to 7.50. In some mice, the electrocorticogram was almost isoelectric (*2*).

Although all 7 examined brain regions were significantly altered, there are still interregional differences that need to be considered: A_1AR availability in the striatum decreased to a higher degree than in other brain regions such as the occipital lobe. The particular vulnerability of the striatum to hypoxia has already been described (*59*), but the exact mechanisms remain unclear. Oxygen saturation was well controllable and controlled, which is why the interindividual deviations in V_T were small (73.0 ± 1.2) and no significant correlations regarding SpO_2 could be found. Future research to determine the interregional differences in the human brain is therefore needed. A discussion of interindividual differences can be found in section 4.1 of the supplemental materials.

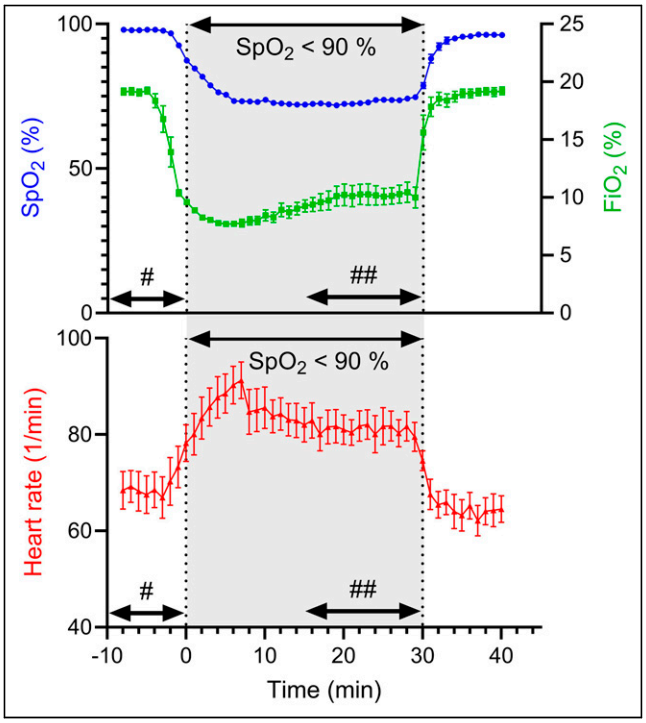

FIGURE 4. Heart rate increased by 19% under hypoxia (P = 0.0039; data are mean ± SEM; 0 min is start of hypoxia). For statistical analysis, normoxia was defined as time before 0 min (#) and hypoxia was for 15–30 min (##). SpO_2 followed inspiratory oxygen fraction (FiO_2) at interval of a few minutes.

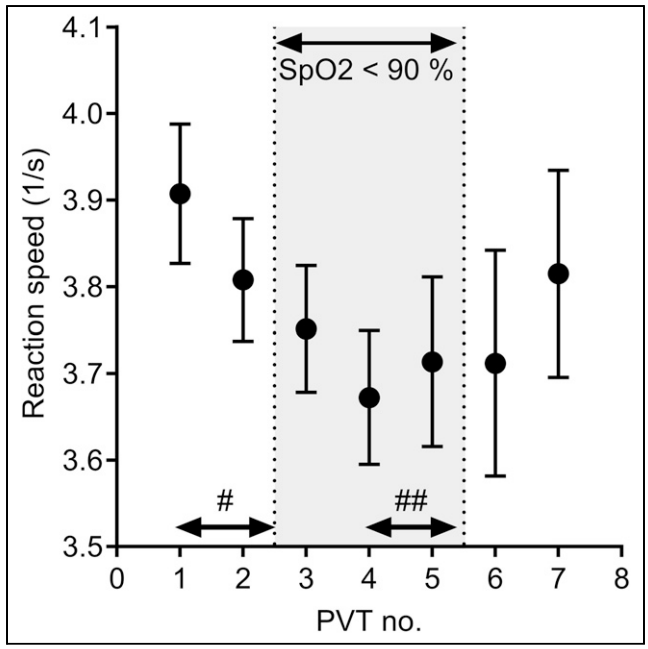

FIGURE 5. PVT showed significant decrease in reaction speed (P = 0.0021; data are mean ± SEM). PVT 1 and 2 were used as normoxia baseline (#), and PVT 4 and 5 were used as hypoxia (##). Each PVT lasted approximately 3 min.

Previous research has demonstrated that insufficient sleep leads to elevated A_1AR availability in humans (*50,56,60*). The experimental study design and use of ActiGraph activity monitors (ActiGraph LLC) ensured that participants were in a well-rested state. Therefore, potential confounding functional effects on A_1AR availability could be eliminated, as well as any potential bias stemming from variations in sleep duration. Since PET imaging was not performed at the same time of day for each subject, we correlated time of day with the relative changes in V_T and found no significant correlations (supplemental materials; Supplemental Table 1). A_1AR availability was shown to decrease with age (*60*); therefore, we included only young subjects with a mean age of 31.3 y, ranging from 19.5 to 46.4 y. Since caffeine displaces up to 44% of [^{18}F]CPFPX binding in a concentration-dependent manner (*61*), subjects had to abstain from caffeine for 1 wk before the study to rule out any potential effect of caffeine use or withdrawal symptoms on receptor occupancy (*62,63*).

One previous study reported that the effects of blood flow exhibit only a small impact on the distribution of PET neuroreceptor tracers (*64*). Therefore, altered blood flow to the brain can be ruled out as the cause of changes in receptor availability. Even in the unlikely event that it did have a small effect, this effect would be opposite to the observed drop in A_1AR availability. Also an increase in blood viscosity known under chronic hypoxia can be excluded in acute hypoxia (*65*), so one can reasonably assume that blood values remained constant.

Our findings are consistent with a hypoxia-induced increase in cerebral adenosine release. Because of low de novo synthesis of adenosine in the brain (*66*), researchers are currently investigating whether the main source of adenosine in nonischemic hypoxia is intracellular or extracellular. There is in vitro and in vivo evidence from mice for both pathways under severe hypoxia (*4*). Future research will be needed to precisely determine the pathways underlying the rise in extracellular adenosine and individual trait characteristics known in other A_1AR-dependent pathways (*51*).

Given adenosine's already-known actions on the brain, A_1AR could contribute to interindividual variability observed in hypoxia tolerance and serve as a target for countermeasures. Our study found a clear correlation between oxygen deprivation and reduced A_1AR receptor availability. However, our results can be only the first step in fully understanding the effects of hypoxia and countermeasures such as caffeine on the human brain (supplemental materials).

Gray Matter Brain Perfusion

We found an increase in gray matter brain perfusion during hypoxia reaching from 27.4% in the striatum to 44.9% in the parietal lobe. A time-resolved ASL technique insensitive to changes in arterial arrival time was used (*48,49,67*), supporting results reported in earlier perfusion studies in hypoxia (*67*).

The ASL sequence we used was particularly robust, using 4 postlabel delays ranging from 0.25 to 1.9 s. Thus, changing the arterial arrival time of blood in the brain did not affect the quality of the data (*48*) and was therefore well suited to the measurements with a mean arterial arrival time of 0.98 s (section 2.4 of the supplemental materials). Furthermore, gray matter perfusion values were consistent across all subjects, showed low variability, and remained in a reasonably expected range.

Heart Rate and Vital Parameters

The supplemental materials provide information on heart rate and vital parameters.

PVT

Psychomotor vigilance in response to hypoxia deteriorated. The average reaction time of the subjects was prolonged by 4.6% (12 ms), and the reaction speed was significantly reduced by 4.3% ($P = 0.0021$). This impairment of reaction time is comparable to a blood alcohol concentration of 0.06% (supplemental materials) (*68*).

Correlation

We correlated the relative changes in A_1AR availability, brain perfusion, and reaction speed to identify individual regions involved in mediating the effects of endogenous adenosine and slower reaction time. Various temporal intervals were assessed using Pearson and Spearman correlation for 0–5, 0–10, 0–15, and 15–30 min. Despite the high resolution of data and the significant relative changes within individual metrics, no statistically significant correlations emerged between the measured variables. Implementing a 10-min PVT and increasing the sample size with measurements at varying oxygen concentrations may help to elucidate potentially existing correlations. Correlations of relative changes in the ASL and V_T values of respective brain regions were not significant (Supplemental Fig. 2).

CONCLUSION

Short-term reduction of oxygen saturation to 70%–75% (corresponding to an oxygen saturation at an altitude of approximately 5,500 m) increases cerebral perfusion and impairs cognitive performance while A_1AR availability is reduced. The finding is consistent with hypoxia-induced cerebral adenosine release leading to increased A_1AR occupancy. Given adenosine's known action as an inhibitory neuromodulator on the human brain, A_1AR could contribute to the variability in hypoxia tolerance and serve as a target for countermeasures against hypoxia.

DISCLOSURE

This research was supported by internal funds from the German Aerospace Center (DLR) and the Research Center Jülich (FZJ). Alexander Drzezga reports the following conflicts of interest: research support from Siemens Healthineers, Life Molecular Imaging, GE HealthCare, AVID Radiopharmaceuticals, SOFIE, Eisai, Novartis/AAA, and Ariceum Therapeutics; speaker honoraria from or advisory board membership with Siemens Healthineers, Sanofi, GE HealthCare, Biogen, Novo Nordisk, Invicro, Novartis/AAA, and Bayer Vital; ownership of stock with Siemens Healthineers, Lantheus Holdings, Structured Therapeutics, and ImmunoGen; and patent EP3765097A1 for ^{18}F-JK-PSMA-7 (January 20, 2021). No other potential conflict of interest relevant to this article was reported.

KEY POINTS

QUESTION: Will short-term reduction of oxygen saturation increase cerebral perfusion and decrease availability of A_1AR?

PERTINENT FINDINGS: In a within-subject–design experimental study with 10 subjects, a short-term reduction in oxygen saturation increased cerebral perfusion and impaired cognitive performance while A_1AR availability was reduced.

IMPLICATIONS FOR PATIENT CARE: Medication acting on the A_1AR could pave the way in developing new therapeutic strategies against brain ischemia.

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Erratum

In the article "[^{18}F]FDG and [^{68}Ga]Ga-FAPI-04–Directed Imaging for Outcome Prediction in Patients with High-Grade Neuroendocrine Neoplasms," by Michalski et al. (*J Nucl Med*. 2024;65:1899–1903), the article title should have been "[^{18}F]FDG and [^{68}Ga]Ga-FAPI-04 Imaging for Outcome Prediction in Patients with High-Grade Neuroendocrine Neoplasms". We regret the error.

4. Diskussion

4.1. A_1AR-Verfügbarkeit

Wir untersuchten die Auswirkungen einer akuten 30-minütigen Exposition gegenüber Hypoxie, die zu einem SpO_2 von knapp über 70 % führte, auf das menschliche Gehirn bei zehn gesunden Probandinnen und Probanden. Die Haupthypothese konnte bestätigt werden, da wir mithilfe des selektiven Tracers [^{18}F]CPFPX[11] eine global reduzierte zerebrale A_1AR-Verfügbarkeit feststellten. Diese reichte von 11,5 % im Okzipitallappen bis 17,9 % im Striatum. Die Ergebnisse konnten auch im Lassen-Plot reproduziert werden, der die Veränderung der A_1AR-Belegung zeigt.[12-14] Da diese Unterschiede in allen untersuchten Hirnregionen beobachtet werden konnten, weist dies auf einen globalen Anstieg des endogenen Adenosins unter hypoxischen Bedingungen hin. Diese Ergebnisse stellen nach unserem Wissen den ersten indirekten Beweis für einen Anstieg des zerebralen Adenosinspiegels während Hypoxie beim Menschen dar. Entsprechende Hypothesen auf der Grundlage von Tiermodellen konnten somit bestätigt werden.[5-7]

4.1.1. Vergleich mit Tiermodell

Im Vergleich zum Tiermodell[5-7] erwarteten wir im Rahmen dieser Studie beim Menschen einen geringeren Anstieg des Adenosins, da die Ratten geringeren normobaren Sauerstoffkonzentrationen (8 bis 9 % gegenüber 10 %) und mehreren Durchgängen von Hypoxie ausgesetzt wurden. Über dies hinaus mussten die Nager mittels Halothan-Narkose ruhiggestellt werden, was die Vergleichbarkeit der beiden Modelle weiter beeinflusste. Durch diese extremeren Bedingungen waren auch die Änderungen der Vitalparameter der Ratten deutlicher: Der systolische Blutdruck fiel von 112 auf 70 mmHg ab, der CO_2-Partialdrucks von 39 auf 25 mmHg, gleichzeitig stieg der pH-Wert von 7,39 auf 7,50. Einige Ratten hatten sogar ein nahezu isoelektrisches Elektrokortikogramm.[6]

4.1.2. Interregionale und interindividuelle Unterschiede

Alle sieben von uns untersuchten Gehirnregionen waren signifikant verändert, dennoch müssen folgende interregionale Unterschiede berücksichtigt werden: Die A_1AR-Verfügbarkeit

im Striatum nahm stärker ab (17,9 %) als in anderen Regionen wie dem Okzipitallappen (11,5 %). Die besondere Anfälligkeit des Striatums für Hypoxie wurde bereits beschrieben,[15] die genauen zugrunde liegenden Mechanismen bleiben jedoch weiterhin unklar. Die Sauerstoffsättigung war gut steuerbar und kontrollierbar (73,0 ± 1,2 % in Hypoxie), weshalb interindividuelle Abweichungen im Verteilungsvolumen V_T keine signifikanten Korrelationen zum SpO_2 zeigten. Zukünftige Forschung zur Bestimmung der interregionalen Unterschiede im menschlichen Gehirn ist daher weiterhin erforderlich.

Neben der interregionalen muss auch die interindividuelle Variabilität berücksichtigt werden: Bei einem einzigen Probanden in unserer Studie stieg das Verteilungsvolumen V_T während der Hypoxie im Gegensatz zu allen anderen Individuen an (siehe Figure 2B). Auch die Herzfrequenz war im Gegensatz zu sämtlichen anderen Probandinnen und Probanden während der Hypoxie nicht erhöht, sondern verringerte sich im Vergleich zum normoxischen Ausgangswert atypisch um 4 % (mittlerer Anstieg der Herzfrequenz bei allen anderen neun Individuen: 21,4 %). Wir gehen davon aus, dass bei diesem Probanden andere Faktoren wie z.B. Aufregung und/oder Angst in der unbekannten Umgebung (MRT, Atemmaske, Lärm, Blutentnahmen, etc.) während der anfänglichen Normoxie-Phase größer waren und daher etwaige spätere Wirkungen des Adenosins durch diese Stimuli und Auswirkungen des Sympathikus überlagert wurden. Eine weitere Möglichkeit ist die äußerst individuelle biochemische Reaktion jedes Menschen auf Hypoxie. Es ist bekannt, dass Hypoxie eine Vielzahl von Reaktionen auslöst, so dass auch eine seltene individuelle Genvariante für diese Reaktion verantwortlich sein könnte.[16] Da wir keinerlei Anzeichen für einen Messfehler sahen, haben wir diesen Probanden nicht von der Analyse ausgeschlossen.

4.1.3. Ausschluss von potenziellen Störfaktoren

Frühere Studien haben gezeigt, dass unzureichender Schlaf zu einer erhöhten Verfügbarkeit von A_1AR beim Menschen führt.[17-19] Durch das Studiendesign und die Verwendung von ActiGraph-Aktivitätsmonitoren (Acti Graph LLC) wurde sichergestellt, dass sich die Probandinnen und Probanden im ausgeruhten Zustand befanden und interindividuelle Unterschiede nicht von unterschiedlichem Schlafverhalten ausgelöst wurden. Daher konnten

potenziell störende Effekte auf die A_1AR-Verfügbarkeit ebenso ausgeschlossen werden wie eine potenzielle Verzerrung aufgrund von Schwankungen der Schlafdauer. Da die Studie nicht bei allen Probandinnen und Probanden zur gleichen Tageszeit durchgeführt werden konnte, korrelierten wir die Uhrzeit der Untersuchung mit den relativen Veränderungen der V_T. Wir fanden keine signifikanten Korrelationen bezüglich der Tageszeit (Supplemental Table 1). Dementsprechend kann eine Auswirkung der Tageszeit auf die Testergebnisse ausgeschlossen werden.

Da die A_1AR-Verfügbarkeit mit dem Alter abnimmt,[19] wurden nur Probandinnen und Probanden mit einem Durchschnittsalter von 31.3 und einer Altersspanne von 19,5 bis 46,4 Jahren inkludiert.

Des Weiteren mussten Probandinnen und Probanden vor der Studie sieben Tage auf Koffein verzichten, da diese psychoaktive Substanz kompetitiv bis zu 44 % der [^{18}F]CPFPX-Bindung verdrängt.[14] Somit wurden mögliche Auswirkungen von Koffeinkonsum oder Entzugserscheinungen auf die gemessene A_1AR-Verfügbarkeit ausgeschlossen.[20,21]

Die von uns festgestellte Änderung der zerebralen Perfusion kann als Störfaktor ausgeschlossen werden, da dies nur einen geringen Einfluss auf die Verteilung von Tracern im Gehirn hat.[22] Selbst für den unwahrscheinlichen Fall, dass der veränderte Blutfluss eine geringe Auswirkung hätte, wäre der Effekt der *gesteigerten* Gehirnperfusion dem beobachteten *Rückgang* der A_1AR-Verfügbarkeit entgegengesetzt. Daher kann eine veränderte Durchblutung des Gehirns als Ursache für die gemessenen Veränderungen der Rezeptorverfügbarkeit definitiv ausgeschlossen werden. Auch ein Anstieg der Blutviskosität – bekannt bei chronischer Hypoxie – kann bei akuter Hypoxie ausgeschlossen werden.[23]

[^{18}F]CPFPX zeigt beim Menschen einen schnellen Metabolismus. Um potenzielle Störfaktoren für die Messung mittels Bolus-Infusion auszuschließen, musste gezeigt werden, dass Hypoxie keinen Einfluss auf den Metabolismus hat. Dies wurde erreicht, indem wir die durchschnittliche metabolitenkorrigierte Plasma-Input-Funktion unserer Hypoxie-Probanden mit den alters- und geschlechts-gematchten Kontrollprobanden der bereits o.g. Studie mit identischem Design von

Pierling et al.[24] verglichen haben (Supplemental Figure 1, V_T-Werte dargestellt in Figure 2 A und B). Trotz des sehr gut erreichten Gleichgewichts in beiden Gruppen werden im Institut für Neurowissenschaften und Medizin (INM) des Forschungszentrums Jülich (FZJ) in der Nuklearchemie (INM-5) weitere Anstrengungen unternommen, einen Radiotracer mit einem langsameren Stoffwechsel zu entwickeln.[25]

4.1.4. Aktuelle und zukünftige Anwendungsgebiete

Aufgrund seiner ubiquitären Verteilung im Gehirn,[4] ist der A_1AR ein wichtiges Ziel für zukünftige therapeutische Interventionen. Ein genaueres Verständnis von Hypoxie und den damit verbundenen Auswirkungen auf den A_1AR könnte den Weg zu wirksameren Interventionen und/oder Medikamenten ebnen. Studien an Mäusen haben gezeigt, dass die Antagonisierung von A_1AR eine protektive Wirkung bei Tauopathien hat und diese sogar rückgängig machen kann,[26] was potenzielle Behandlungen der Alzheimer-Krankheit möglich macht. Eine andere Studie an Ratten zeigte die schützende Wirkung von Koffein bei neonataler Hypoxie, indem es den Verlust von Gehirnarealen verhindert.[27] Als die am häufigsten verwendete psychoaktive Substanz weltweit[28] kann Koffein durch die Hemmung des A_1AR die kurzfristige Leistungsfähigkeit unter nicht-ischämischen hypoxischen Bedingungen verbessern. Es gibt Studien, die die Sicherheit der Einnahme von Koffein unter moderaten hypoxischen Bedingungen in großer Höhe belegen und zeigen, dass Bedenken hinsichtlich Dehydrierung[29] und erhöhtem Blutdruck[30] höchstwahrscheinlich übertrieben sind. Für gewohnheitsmäßige Koffeinkonsumenten ist es sogar gefährlich, den Koffeinkonsum in der Höhe einzustellen, da die Entzugserscheinungen denen der akuten Höhenkrankheit ähneln.

Koffein kann auch geistige und körperliche Leistungsfähigkeit unter hypoxischen Bedingungen verbessern.[29,31] Die erhöhte Leistungsfähigkeit ist nicht nur bei der objektiven Erschöpfung messbar, sondern auch bei der subjektiven Wahrnehmung von Müdigkeit.[31] Erhöhte Adenosinspiegel beeinträchtigen somit nicht nur die psychomotorische Wachsamkeit, sie sind auch für die Wahrnehmung von Leistungsfähigkeit und Müdigkeit von entscheidender Bedeutung.

Diese Wahrnehmung wird regelmäßig von finnischen Militärpiloten trainiert, die in Hypoxie die Auswirkungen von Sauerstoffmangel auf die Wahrnehmung und Informationsverarbeitung erkennen müssen. Dieses regelmäßige Training schärft das Bewusstsein für potenziell gefährliche Episoden von Hypoxie im Cockpit.[32] Die erhöhte Aufmerksamkeit aus dem ersten Training hält dabei bis zu 2,4 Jahre an. Diese langanhaltende Verbesserung der Wahrnehmung von Hypoxie-Symptomen unterstreicht die Notwendigkeit umfassenderer Studien, um die genaue Rolle von Adenosin bei der Modulation von Hypoxie und ihrer Wahrnehmung aufzuklären.

Hypoxie kann auch als potenzielle Therapie bei verschiedenen Krankheiten eingesetzt werden. Es gibt eine Vielzahl explorativer Untersuchungen, die vielseitiges therapeutisches Potenzial von moderatem Sauerstoffmangel aufzeigen: Hypoxie reguliert hämatologische, metabolische, angiogene und neurale Anpassungen. Bei Darmkrebs hat systemische Hypoxie in Kombination mit Bewegung nicht nur positive prognostische Auswirkungen, sondern verbessert auch die Lebensqualität.[33] Darüber hinaus kann intermittierende Hypoxie über einen Zeitraum von 24 Wochen auch präventiv eingesetzt werden, um die Körperfettmasse positiv zu beeinflussen, Entzündungsparameter zu senken und Marker des Knochenumbaus zu verbessern.[34] Die Kombination von Hypoxie und Krafttraining führt zu Schwankungen der Kalzium-, Phosphat-, Bikarbonat-, Cortisol- und Wachstumshormonspiegel.[35] Die genauen physiologischen Mechanismen hinter diesen Veränderungen sind weiterhin ungeklärt, sie scheinen jedoch nicht auf Veränderungen in der Hypothalamus-Hypophysen-Achse zurückzugehen, sondern auf Erschöpfung und Laktatanhäufung während des Trainings.[36]

Weiteres therapeutisches Potenzial erklärt sich aus den sozioökonomischen Auswirkungen von ischämischen Schlaganfällen, welche 87 % aller Schlaganfälle ausmachen[37] und durch einen lokalen Sauerstoffmangel im Gehirn gekennzeichnet sind. Es wurde im Mausmodell gezeigt, dass A_1AR und seine partiellen Agonisten die Wiederherstellung der synaptischen Übertragung nach einem ischämischen Schlaganfall erheblich unterstützen.[38] Der A_1AR spielt außerdem eine zentrale Rolle bei der Aktivierung, Proliferation und Entzündung von Gliazellen

nach einem experimentellen Schlaganfall bei Ratten.[39] Dies zeigt die vielen möglichen Anwendungen zukünftiger Medikamente. Weitere Forschung ist erforderlich, um diese potenzielle Behandlung von ischämischen Schlaganfällen besser verstehen zu können. Die jährlichen Kosten dieses Krankheitsbildes in Europa belaufen sich auf etwa 60 Milliarden Euro[40], zudem ist der Schlaganfall die häufigste Ursache für erworbene Behinderungen im Erwachsenenalter in Deutschland.[41] Aufgrund der alternden Bevölkerung ist eine wirksamere Behandlung in Zukunft von entscheidender Bedeutung.

4.1.5. Zusammenfassende Einordnung der A_1AR-Messwerte

Die Ergebnisse der vorliegenden Studie lassen sich mit einem hypoxiebedingten Anstieg der zerebralen Adenosinfreisetzung erklären. Aufgrund der geringen de-novo-Synthese von Adenosin im Gehirn[42] wird derzeit untersucht, ob die Hauptquelle von Adenosin bei nicht-ischämischer Hypoxie intrazellulär oder extrazellulär liegt. Es konnten bei schwerer Hypoxie in Mäusen für beide Pathways in-vitro- und in-vivo-Nachweise erbracht werden.[7] Zukünftige Forschung sollte die dem Anstieg des extrazellulären Adenosins zugrunde liegenden Wege und individuelle Merkmale genau bestimmen, die bei anderen A_1AR-abhängigen Wegen bereits bekannt sind.[43]

Die bekannte Wirkung von Adenosin auf das Gehirn lässt darauf schließen, dass der A_1AR zur interindividuellen Variabilität beiträgt, die bei Hypoxietoleranz beobachtet wird. Somit könnte er auch als Ziel für Gegenmaßnahmen dienen. Unsere Studie ergab eine klare Korrelation zwischen Sauerstoffmangel und einer verminderten Verfügbarkeit des A_1AR-Rezeptors. Die Ergebnisse können jedoch nur ein erster Schritt sein, um die Auswirkungen von Hypoxie und Gegenmaßnahmen wie Koffein auf das menschliche Gehirn vollständig zu verstehen.

4.2. Perfusion des Gehirns

Die Perfusion der grauen Substanz des Gehirns stieg in Hypoxie von 27,4 % im Striatum bis zu 44,9 % im Parietallappen an. Es wurde eine zeitlich hochauflösende ASL-Sequenz

verwendet, die unempfindlich gegenüber Änderungen der Arterial Arrival Time (AAT) ist.[44-46] Die Ergebnisse früherer Perfusionsstudien bei Hypoxie wurden somit bestätigt.[44]

Die von uns verwendete ASL-Sequenz war besonders robust und gut für unseren Versuchsaufbau geeignet, da sie vier Postlabel-Delays (PLDs) im Bereich von 0,25 bis 1,9 s verwendete. Daher hatte eine durch Hypoxie verursachte Änderung der AAT im Gehirn keinen Einfluss auf die Qualität der Daten.[45] Die mittlere AAT unserer Individuen lag bei 0.98 s und lag somit mitten im Messbereich der PLDs (siehe Methods der Publikation).

Zusätzlich zur gesteigerten Perfusion des Gehirns in Hypoxie, konnte auch eine Verkürzung der AAT um 9,6 % von 1,04 s in Normoxie auf 0,94 s in Hypoxie beobachtet werden. Somit wird das Gehirn während akuter Hypoxie nicht nur besser perfundiert, auch wird das Blut dem Gehirn schneller zur Verfügung gestellt.

4.3. Herzfrequenz und Vitalparameter

Die Herzfrequenz stieg unter hypoxischen Bedingungen signifikant um 19 % an (P = 0,0039). Die Arterialisierung des Bluts des linken Arms durch Erwärmung mit einer Heizmatte sorgte für zuverlässigere Messungen, da die SpO_2-Sensoren aufgrund der gesteigerten Durchblutung weniger fehleranfällig waren. Aufgrund des Studiendesigns und des geplanten Absinkens der peripheren Sauerstoffsättigung auf knapp über 70 %, waren wir auf zuverlässige Messungen der Vitalparameter angewiesen. Daher wurde zusätzlich am anderen Arm der Siemens-interne Sauerstoffclip verwendet. Zusammen mit den EKG-Daten standen damit drei Quellen für die Pulsdaten und zwei für die periphere Sauerstoffsättigung zur Verfügung. Der Verlauf des SpO_2 und der geringe Standardfehler in Figure 4 zeigen auch, wie gut die Hypoxie in unserem Studiendesign kontrolliert werden konnte. Dies unterstreicht die Präzision und Zuverlässigkeit der Messergebnisse.

Der bereits beschriebene Anstieg der Herzfrequenz unter Hypoxie[9] wurde von uns bestätigt. Darüber hinaus konnten wir aufgrund der hohen zeitlichen Auflösung unserer Datenerfassung

den Verlauf und die Geschwindigkeit der Anpassung der Herzfrequenz an akute Hypoxie beobachten. Aufgrund der mit zunehmendem Alter abnehmenden Toleranz gegenüber Ischämie und Hypoxie,[47] inkludierten wir ausschließlich jüngere Probandinnen und Probanden im Alter von 19 bis 46 Jahren.

Mit Einsetzen der Hypoxie stieg die Herzfrequenz innerhalb von zwei Minuten rapide an. Dieser Pulsanstieg erfolgte schneller als die Veränderung der A_1AR-Verfügbarkeit und war vergleichbar mit der Geschwindigkeit der Veränderung der Hirnperfusion. Noch bevor die mittels Fingerclip gemessene SpO_2 unter 90 % fiel, reagierte der Körper auf den Abfall der inspiratorischen Sauerstofffraktion zu Beginn der Hypoxie mit einem schnellen Anstieg der Herzfrequenz. Nach Erreichen eines Plateaus nach etwa fünf Minuten Hypoxie, blieb die Herzfrequenz bei allen Probandinnen und Probanden konstant erhöht. Als die Sauerstoffversorgung wieder normale 21 % betrug, sank die Herzfrequenz rapide auf das Ausgangsniveau zurück, sogar noch bevor die SpO_2 wieder auf normoxische Werte angestiegen war.

4.4. Psychomotor Vigilance Task (PVT)

Die psychomotorische Vigilanz der Probandinnen und Probanden verschlechterte sich in Hypoxie. Die durchschnittliche Reaktionszeit verlängerte sich um 4,6 % (12 ms), und die Reaktionsgeschwindigkeit verringerte sich signifikant um 4,3 % (P = 0,0021). Diese Beeinträchtigung der Reaktionszeit ist vergleichbar mit einer Blutalkoholkonzentration von 0,06 ‰.[48]

Diese durch Hypoxie verursachte Abnahme der Reaktionsgeschwindigkeit und die Rückkehr zum Ausgangswert in Normoxie, fanden im Vergleich zu den Änderungen der Herzfrequenz und Perfusion langsamer statt. Dies deutet darauf hin, dass komplexere Funktionen des Gehirns, wie die Reaktionsgeschwindigkeit und psychomotorische Vigilanz, die von der Verarbeitung in verschiedenen Hirnregionen abhängig sind, träger auf hypoxische Reize reagieren als schnelle physiologische Regelkreise wie Perfusion oder Herzfrequenz.[49]

Aufgrund dieser zu erwartenden Trägheit, analysierten wir Daten aus der zweiten Hälfte der Hypoxie zum Vergleich mit Normoxie (siehe 2.8 im Supplement). Da alle zehn Minuten eine Blutprobe entnommen wurde, wurde eine 3-Minuten-Version des PVT verwendet, da sie in anderen Studien nachweislich zuverlässige Ergebnisse erzielt hat.[10,50] Dadurch wurde eine Überschneidung von PVT und Blutentnahme verhindert.

Trotz signifikanter Ergebnisse sowohl beim PVT als auch bei der A_1AR-Verfügbarkeit führte ein Test der Korrelation beider Werte nicht zu einem statistisch signifikanten Ergebnis (siehe 4.5). Allerdings wurden sowohl der 3-minütige- als auch der 10-minütige-PVT für die Beurteilung von Schlafmangel und alkoholbedingten Beeinträchtigungen der kognitiven Leistung validiert[10] und könnten daher für Veränderungen in Hypoxie zu rudimentär und simpel sein. Eine multidimensionale Beurteilung wie der d2-Test, der komplizierte kognitive Prozesse bewertet, könnte korrelierbare Ergebnisse liefern.[51]

Zur Steigerung der psychomotorischen Vigilanz konsumieren viele Menschen täglich Koffein, um die durch Adenosin vermittelte Müdigkeit am A_1AR zu antagonisieren. Unter hypoxischen Bedingungen wirkt Koffein aber nicht nur der tatsächlich messbaren objektiven Erschöpfung entgegen, sondern auch der subjektiven Wahrnehmung von Müdigkeit.[31] Dies weist darauf hin, dass erhöhte Adenosinspiegel die psychomotorische Wachsamkeit und damit das Empfinden von Hypoxie beeinflussen.

4.5. Korrelation

Zwischen relativer Veränderung der A_1AR-Verfügbarkeit, Gehirnperfusion und Reaktionsgeschwindigkeit wurde auf Korrelation getestet, um einzelne Regionen zu identifizieren, die an der Vermittlung der Auswirkungen von endogenem Adenosin und der langsameren Reaktionszeit beteiligt sind. Unter Verwendung der Pearson- und Spearman-Korrelation wurden jeweils die zeitlichen Intervalle 0 bis 5, 0 bis 10, 0 bis 15 und 15 bis 30 min untersucht. Damit wurden auch die akuten Zeiträume direkt nach Beginn der Hypoxie abgedeckt. So konnten wir auch Korrelationen während der Anpassungsphase an Hypoxie

untersuchen. Trotz der hohen zeitlichen Auflösung der einzelnen Datensets und der jeweils signifikanten relativen Veränderungen, ergaben sich keine statistisch signifikanten Korrelationen zwischen den gemessenen Variablen.

Die Durchführung eines 10-minütigen PVT, die Erhöhung der Stichprobengröße und/oder Messungen bei unterschiedlichen Sauerstoffpartialdrücken könnten dazu beitragen, potenziell vorhandene Korrelationen aufzuklären. Auch die Korrelationen der relativen Veränderungen der ASL- und V_T-Werte der jeweiligen Hirnregionen zeigten keine signifikanten Ergebnisse (Supplemental Figure 2).

4.6. Zusammenfassende Diskussion

Eine kurzzeitige Reduktion der Sauerstoffsättigung auf 70 % bis 75 % (entsprechend einem Sauerstoffpartialdruck der Atemluft auf etwa 5.500 m) steigert die zerebrale Perfusion und beeinträchtigt die kognitive Leistungsfähigkeit, während gleichzeitig die A_1AR-Verfügbarkeit reduziert ist. Nach unserem Wissen stellt dies die erste Studie dar, welche die verringerte A_1AR-Verfügbarkeit unter Hypoxie im menschlichen Gehirn darstellt.

Diese Ergebnisse lassen sich mit einer Hypoxie-induzierten Ausschüttung von Adenosin im menschlichen Gehirn und einer dadurch erhöhten Belegung des A_1AR vereinbaren. Aufgrund der bereits bekannten inhibitorischen neuromodulatorischen Eigenschaften von Adenosin, könnte der A_1AR zur Variabilität der Hypoxietoleranz beitragen und für zukünftige Erforschung von Gegenmaßnahmen entscheidend sein.

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6. Anhang

6.1. Abbildungsverzeichnis

6.2. Tabellenverzeichnis

6.3. Supplement

Das beim Journal of Nuclear Medicine mit dem Artikel zusammen veröffentlichte Supplement ist unter https://jnm.snmjournals.org/content/66/1/142/tab-supplemental abrufbar und diesem Dokument angehangen.

Supplemental Data

Methods

2.1 Study protocol

Exclusion criteria were as follows: chronic neurological or psychiatric disorders, head trauma, sleep disorder, shift or night work, alcohol and drug abuse, smoking, pregnant or breast-feeding females. Only participants reporting no current medication (except contraceptives) and an estimated habitual caffeine consumption below 450 mg/day were included in the present investigation. A physical examination was carried out before the start of the study by a medical doctor of the German Aerospace Center.

Participants had to abstain from alcohol and caffeine one week before the PET scan. All participants reported their habitual sleep behavior on working days and followed a one-week ambulatory sleep satiation protocol (nine hours time-in-bed (TIB); 10:00/11:00 p.m.–07:00/08:00 a.m.), which was verified by actigraph recording and sleep diaries. On the study day participants' urine was tested for the following substances: cotinine, zolpidem, propoxyphene, amphetamines, barbiturates, benzodiazepines, cocaine, methamphetamines, heroin, morphine, methadone, ecstasy, tricyclic antidepressants, and tetrahydrocannabinol.

The study was conducted in the PET-MRI facility at :envihab of the German Aerospace Center in Cologne (https://www.dlr.de/envihab/).

2.2 Breathing gas mixture

A quickly adjustable mixture of the breathing gas was achieved by combining pressurized air (21 % oxygen concentration) and nitrogen enriched pressured air (8 % oxygen concentration, 13 % higher nitrogen concentration than in normal breathing air) using a Bird company standard blender approved for clinical use. The gas mixture was delivered via a tube to a reservoir bag right next to an airtight breathing mask. The reservoir bag compensated for the high peak flows of inspiration. Due to the volume of the reservoir bag and compensation mechanisms (*7*) participants' mean time to achieve hypoxia (onset defined by $SpO_2 < 90\%$) after administering nitrogen-enriched pressured air was approximately 2.5 min.

Between reservoir bag and breathing mask, the oxygen fraction of the air was monitored in real time with a Servomex ServoPro MultiExact. Expiration was monitored via CO_2 flow meter, such that breathing rate could be measured.

2.3 PET/MRI data acquisition

For PET calibration and normalization, a 68 Ge/68 Ga phantom was employed daily. Additionally, an aliquot of the 68 Ge/68 Ga phantom was subjected to gamma counting (Wizard 2; PerkinElmer) to determine the cross-calibration factor.

2.4 Arterial Spin Labeling (ASL)

We acquired perfusion images with an average duration of 8.5 min before hypoxia, 30 min during hypoxia and 15 min after hypoxia. With a label-acquisition cycle lasting for 1 min 22 s resulting in on average 6 cycles before, 22 during and 11 after hypoxia.

In total 16 proton density weighted images with a repetition time of 6 s with both phase encoding polarities were acquired before the ASL images to calibrate perfusion images. Additional parameters included: repetition time/echo time 10260.0 ms/22.9 ms, CAIPIRINHA R = 2 × 2 (Δ = 1), 3.3 mm isotropic resolution, 211 mm × 211 mm field-of view (FOV), 36 slices, single-shot acquisition with band width (BW) = 2367 Hz/Px, EPI factor 32, turbo factor (TF) 18 (similar to the parameters chosen in (*8*))

Data processing and -analysis:

Each structural T1 scan was preprocessed using the FSL_ANAT Processing Script of the FSL library (*9*). The following steps were executed: reorientation of the images to the standard (MNI) orientation (with fslreorient2std), automatic cropping of the image (with robustfov), bias-field correction (with FAST), registration to standard space (with FLIRT and FNIRT), brain-extraction (with BET), tissue-type segmentation (with FAST) and subcortical structure segmentation (with FIRST).

Analysis of ASL data was performed using Basil (*10*). Voxel-wise calculation of gray matter perfusion and arterial arrival time (AAT) was based on a Bayesian inference method. Proton weighted images were used for estimation of tracer concentration (equilibrium magnetization of arterial blood) and therefore used for calibration of perfusion values. Additionally our analysis included distortion correction (with calibration images in a different phase encoding direction), motion correction (using mcflirt), incorporation of uncertainty in T1 values (including T1 values in the inference process), arterial/macro vascular signal correction (*11*), and partial volume correction (*12*).

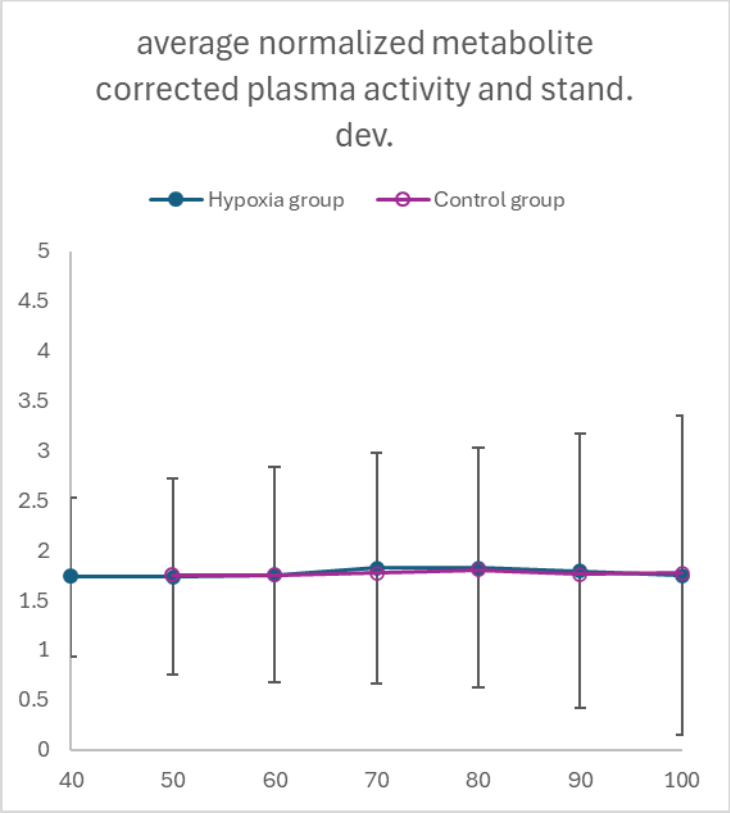
Subsequently, a regional analysis was performed using the registration from the structural image to MNI152 standard space. Regions were defined by the AAL-merged atlas. We evaluated the same brain regions as in our PET data analysis. The defined volumes of interest (VOI) were: frontal lobe, temporal lobe, parietal lobe, occipital

lobe, insula, cingulate cortex, and striatum. Voxels with at least 80 % gray matter were defined as gray matter und used for estimation of gray matter perfusion.

2.5 [^{18}F]CPFPX PET data acquisition and processing

The mean injected dose of [^{18}F]CPFPX was 208.3 ± 39.1 MBq (range 149 – 280 MBq) corresponding to 2.70 ± 0.16 MBq/kg injected activity per bodyweight. The molar activity at the time of injection was 60.88 ± 38.20 GBq/µmol (range 18.87 – 133.87 GBq/µmol). The corresponding mass of [^{18}F]CPFPX injected averaged 5.00 ± 3.04 nmol (range 1.11 – 9.96 nmol).

This bolus plus constant infusion led to an equilibrium of the tracer throughout the experiment and especially during hypoxia (supplemental figure 1).



Supplemental Figure 1: average metabolite corrected plasma activity. Data of the control group were normalized (scaled) to the average activity of the hypoxia group for visualization purposes

PET signal reconstruction process utilized the e7 tools (Siemens Molecular Imaging) and employed the OP-OSEM reconstruction algorithm with point spread function modeling. The reconstruction procedure consisted of three iterations and 21 subsets. For post-filtering, a three mm Gaussian filter was applied. The framing scheme used was 4 × 60 s, 3 × 120 s, and 18 × 300 s. This resulted in matrix dimensions of 344 × 344 × 127, with a reconstructed image resolution of 2.09 × 2.09 × 2.03 mm³. Prior to reconstruction, the images were corrected for detector

normalization, randoms, and scatter. The vendor-supplied UTE sequence was used to generate MRI-based attenuation maps, in which bone, soft tissue, and air are segmented and assigned specified discrete attenuation values (bone: 0.1510 cm^{-1}, soft tissue: 0.1 cm^{-1}, air: 0 cm^{-1}).

Assessment of receptor availability employed an equilibrium pharmacokinetic model. In circumstances of steady-state, the diffusion of the radioligand between the blood plasma compartment (administered through the computerized infusion pump) and the brain tissue compartment (the region "behind" the blood–brain barrier) yields a temporally constant ratio between these compartments. This ratio signifies the distribution volume (V_T), which is directly correlated with the receptor density. During the equilibrium phase of [^{18}F]CPFPX bolus/infusion experiments, venous and arterial concentrations equalize, allowing venous blood sampling to replace arterial sampling (*13, 54*). To achieve optimal blood drawing conditions, the left forearm was heated to 38°C, so arterialized venous blood samples could be collected 2, 40, 50, 60, 70, 80, 90 and 100 min after the start of the [^{18}F]CPFPX infusion. All blood samples were analyzed collectively immediately after completion of the PET scan. Whole blood samples (500 µl) were counted for 120 s in a cross-calibrated γ-counter. Blood samples were centrifuged (3000 g, 3 min) to obtain plasma. Plasma samples (400 µl) were mixed with an extraction solution (acetonitrile/methanol 50/50 v/v, 400 µl), shaken for 60 s at room temperature, counted in duplicate in the γ-counter and then centrifuged at 18 °C (20000 g, 2 min). Aliquots (3 × 5 µl) of the supernatants were applied to a precoated thin-layer chromatography (TLC) plate (809.022; Macherey-Nagel) and developed with an ethyl acetate/heptane 75/25 (v/v) mobile phase to analyze unmetabolized [^{18}F]CPFPX. The pellets were measured in a γ-counter in duplicate. The TLC plates were exposed for three to five hours on imaging plates of type HCR (HR2025cm113; Dürr NDT). The imaging plates were scanned with an imaging plate reader (CR 35 Bio Plus; Dürr Medical) and analyzed with the AIDA Imaging Analysis software (Elysia Raytest). The whole blood, plasma and pellet radioactivity was decay corrected to the beginning of the scan. The time courses of the fraction of total radioactivity extraction relative to the 2-minute sample and the fraction of parent compound in plasma were fitted by nonlinear regression analyses (*13,14, 54*). These adjustments were used to generate metabolite- and extraction-corrected plasma input functions.

PET processing and data analysis:

PET and corresponding MRI data were preprocessed using PMOD Neuro Tool (version 4.1; PMOD Technologies), following the methodology previously described (*55*).

Kinetic modeling was performed using PMOD Kinetics Tool. Regional time-activity curves (TACs) were calculated for each voxel of interest (VOI). A decay-corrected whole blood function and a decay-, metabolism-

and extraction-corrected plasma input function were used to correct the regional TACs. The corrected TACs were used to estimate metabolite-corrected steady-state A_1AR distribution volumes (V_T). Distribution volume at equilibrium (between 40 and 100 min) corresponds to the ratio of the radioligand concentration in the target region in the tissue (C_T; kBq × cm^{-3}) to the plasma activity (C_p; kBq ×mL^{-1}) ratio ($V_T = C_T/C_p$; (*56*)).

PET data were motion-corrected using a reference image created by averaging the PET data from the first nine minutes of the scan. Matching parameters were left at default values, including the squared difference sum cost function, trilinear interpolation, and six mm full width at half maximum (FWHM) smoothing. The T1-weighted MR images were segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF). For this purpose, the MR images were denoised at medium intensity and then segmented using variant 6 probability maps (SPM12). The sampling parameter was set to 3.0 mm, bias regularization compensated for slight modulations in image intensity across the entire field of view, and variations were smoothed with 60 mm FWHM. The cleanup setting was set to thorough, and affine regularization was initialized according to European brains. Post-processing segmentation was performed using a background probability of 0.2 and the superposition strategy with thresholds for the GM and WM probability maps of 0.1 and 0.05, respectively. If PET-MR matching was required, rigid matching was performed based on the normalized mutual information criterion with a matching sampling of 3.0 pixels. Spatial normalization was performed by a probability map transformation using the normalization results of the previous MR segmentation. Seventy volumes of interest (VOIs) were defined by the automatic anatomical labeling template in the Montreal Neurological Institute space implemented in the PMOD software (*15*), to examine hypoxia and cognition specific effects we used the following seven atlas regions: Frontal lobe, temporal lobe, occipital lobe, parietal lobe, striatum, insula, and cingulate gyrus. The PET images were analyzed in atlas space, and the GM probability information was taken from the mask resulting from the segmentation. The boundaries of the cortical VOIs were checked and manually adjusted to avoid false detection of signals from the brain sinuses. (*16,17, 60*).

2.6 Vital parameters

A four-channel electrocardiogram was acquired. Heart rate was documented, ECG was monitored via the Philips Expression MR400. In addition, oxygen saturation was measured on both hands using one wired and one wireless device (see section 2.7.2). The heart rate measured independently by each of these devices was compared with the output from the ECG. Thus, we achieved a reliable measurement of the heart rate.

The wired SpO_2 sensor on the left hand was connected to the WinDaq data acquisition software (WinDaq Pro+ DI-720 Acquisition), which stored heartbeat and oxygen saturation once per second. In addition, we manually noted information from the three parallel measurements once per minute.

On the right-hand side the wireless Siemens MRI SpO_2 sensor, on the left-hand side a Nonin cable connected SpO_2 sensor. The left forearm was also heated to 38°C to arterialize the blood for the venous blood draw (see section 2.6). The increased perfusion of the arm led to a reliable measurement of SpO_2. The mean value of both measurements was used for the statistical evaluation.

We recorded oxygen saturation once per minute.

2.7 Psychomotor vigilance test (PVT)

During the test, subjects had to respond to a white digital stopwatch appearing in irregular intervals (1 to 5 s) on a dark screen by pressing a button as fast as possible. After their reaction, the immediate feedback of the reaction time was displayed on a screen. The test lasted three minutes. We conducted the test seven times every ten minutes, two times before hypoxia, three times during hypoxia and two times after hypoxia. In this study, the number of presented signals averaged 42.5 ± 1.7 per three-min trial. Reaction times equal to or exceeding 500 ms, were categorized as lapses and not used for statistical analyses (*18*). Additionally, reaction times shorter than 130 ms were considered to likely represent reactions without stimulus (false starts) and were consequently excluded from the analysis.

2.8 Statistical analyses

"Normoxia" was defined as the time before the onset of hypoxia (minutes -15 to 0, Figure 1). We assume that a wide variety of mechanisms and effects overlap in the acute phase after hypoxia due to various compensation mechanisms (*7*). Therefore, we did not use the time shortly after returning to normoxic conditions for statistical analysis (minute 30 to 50, Figure 1). Specifically, we used the measurement performed 20 min (PET), seven min (ASL), eight min (heart rate) and two PVTs before the onset of hypoxia to determine the mean normoxic values.

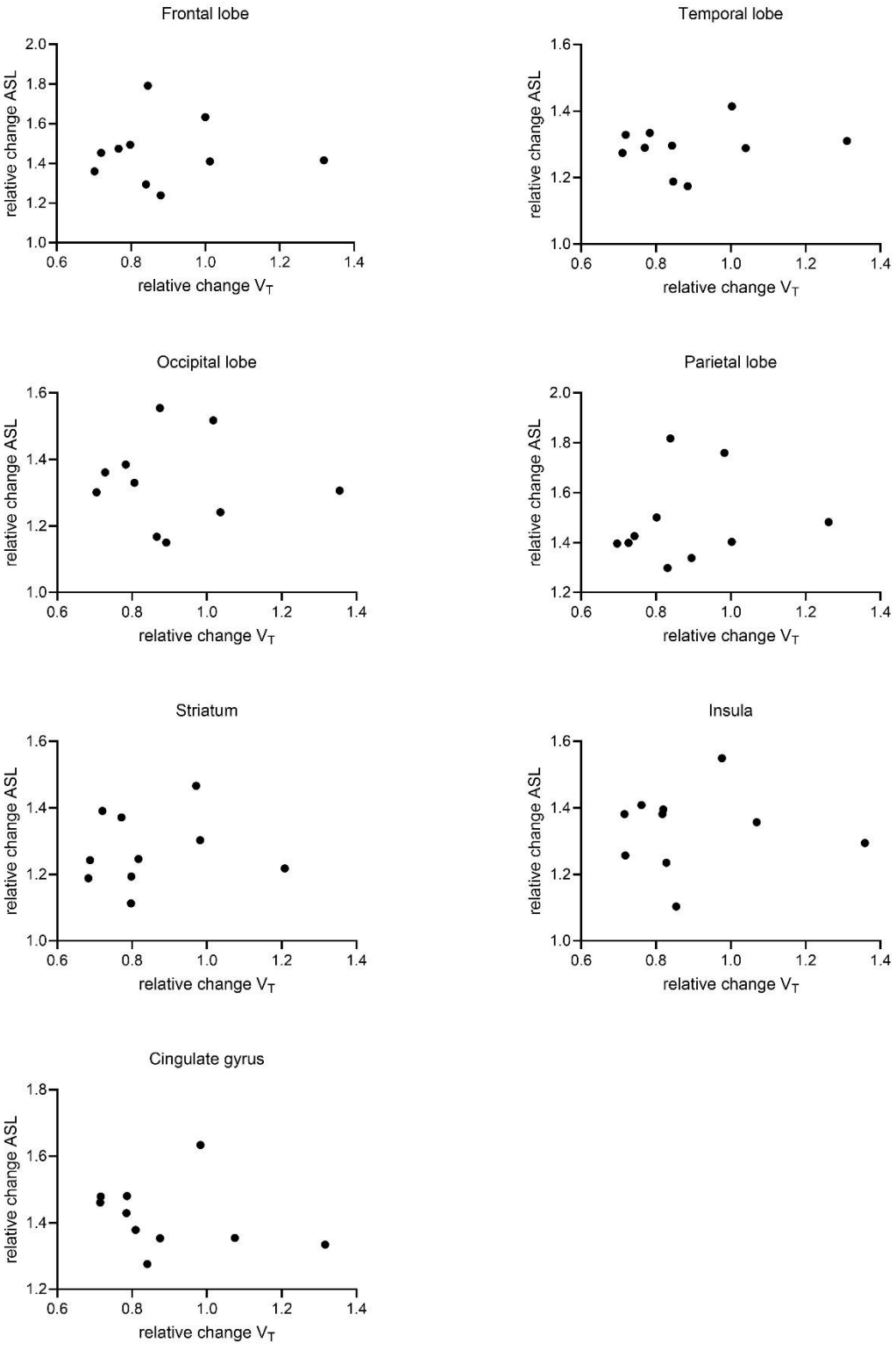
For the same reason of compensation mechanisms, we only used the second half of hypoxia (minutes 15 to 30 after onset of hypoxia) for calculating the mean values for "hypoxia". Specifically, we used the measurement performed min 15 to 30 of hypoxia for PET, ASL, and heart rate and the 4^{th} and 5^{th} PVT to determine the mean hypoxic values. We calculated the relative change in hypoxia compared to normoxia of each subject for each modality.

2.10 Estimation of A_1AR occupancy by Lassen plot

A_1AR occupancy was estimated as previously described in (*61*) and based on steady-state distribution volume in normoxia ($V_{T, Normoxia}$; -20 to 0 min) and distribution volume in hypoxia ($V_{T, Hypoxia}$; 15 to 30 min) (n = 10). The Lassen plot was used to estimate occupancy by plotting the difference in distribution volume between normoxia and hypoxia ($V_{T, Normoxia} - V_{T, Hypoxia}$) versus distribution volume at normoxia ($V_{T, Normoxia}$), with the slope of the regression line corresponding to the occupancy (*19,20*).

Results

3.5 No significant correlation between relative change in distribution volume (VT), perfusion and psychomotor vigilance



Supplemental Figure 2: Scatter plots of relative change of V_T and relative change of ASL for each brain region

See supplemental table 1 and 2 for more detailed correlations.

Discussion

4.1 A_1AR availability

Our main finding was a globally reduced cerebral A_1AR availability. However, inter-individual differences prevail. In a single subject distribution volume rose during hypoxia unlike in any other subject (see Figure 2B). Also, unlike any other subject, the heart rate was not increased during hypoxia but decreased by 4% compared to baseline (mean increase of heart rate of the other 9 subjects: 21.4%).

We assume that in this subject other factors such as arousal/anxiety during the initial normoxia phase were greater and therefore any effects of the adenosine could be overruled by these stimuli. Another possibility is the extremely individual chemical reaction to hypoxia by every human being. Hypoxia is known to trigger a wide variety of reactions and therefore a rare individual gene variant could also be responsible for this reaction. As we saw no evidence of measurement error, we did not exclude the subject from analyses.

CPFPX shows a rapid metabolism in humans. In order to rule out a potential confounder for the quantification of a bolus-infusion setup we confirmed that hypoxia had no influence on the metabolism by comparing the average metabolite corrected plasma input function of the hypoxia subjects with the age and gender matched control subjects of a study with an identical design (supplemental figure 1, V_T values are displayed in figure 2 A and B). Despite the very well achieved equilibrium in both groups, efforts are being made in our group to develop a radiotracer with a slower metabolism (*21*).

Deeper understanding of hypoxia and its effects on A_1AR could lead to better interventions and/or medications in the future. Mouse studies have shown that antagonizing A_1AR has a protective effect in tau pathologies and can even reverse them (*22*), which is promising for treatment of Alzheimer's disease. Another mouse study showed the protective effect caffeine has in neonatal hypoxia by preventing brain area loss (*23*). As the most widely used psychostimulant (*24*), caffeine's inhibition of A_1AR activity has the potential to enhance short term performance under non-ischemic hypoxic conditions. There are studies supporting the safety of caffeine use in high altitude hypoxic conditions, demonstrating that concerns over dehydration (*25*) and increased blood pressure (*26*) are likely overstated. For habitual caffeine users it is even dangerous to discontinue caffeine, because symptoms of withdrawal are similar to acute mountain sickness. In addition there is evidence that caffeine improves mental and physical performance in hypoxic conditions (*25,27*). Interestingly, caffeine administration in hypoxic conditions counteracts not only actual exhaustion but also the perception of fatigue, corroborating our hypothesis that increased adenosine levels impair psychomotor vigilance.

To enhance their ability to manage the effects of both caffeine and oxygen deficiency on perception and information processing, Finnish military pilots regularly undergo hypoxia training. This practice enhances their awareness of potentially dangerous episodes of hypoxia in the cockpit, leading to improved safety measures (*28*). Notably, the heightened awareness from the initial training persists for up to 2.4 years. This long-lasting improvement in the perception of hypoxia-symptoms emphasizes the need for more comprehensive studies to elucidate the precise roles of adenosine and/or caffeine in modulating hypoxia and its perception.

In in vitro investigation (*5*) we found for the binding of titrated CPFPX that IC50 values for caffeine were in the range of 113 to 170 µM and for adenosine of 4.5 to 10.6 µM. An oral uptake of caffeine of 5–8 mg/kg bodyweight (approximately three cups of coffee) leads to plasma peak levels of 8–10 mg/L (*29,30*). As the plasma-to-cerebrospinal fluid ratio at equilibrium conditions is about 1 for caffeine (*31*), concentrations can be assumed to be in the range of 50 µM. Estimations of resting adenosine levels based on microdialysis experiments in the cat brain were 150–300 nM (*32*), in the rat brain 30–300 nM and 100–130 nM in the human brain (in epileptogenic tissue before surgery) and increases were observed to be several-fold under hypoxia in rodents. Adenosine and caffeine displaced [3H]CPFPX completely while adenosine was approximately 20 times more potent than caffeine. A hypothetical estimation based on those numbers (adenosine levels 0.2 µM x 5 (hypoxia effect): 1 µM x 20 (potency factor): 20 µM as caffeine equivalent concentration) would conclude that the hypoxia-induced reduction would be smaller than previously observed caffeine-induced changes.

The cerebellum is known to have one of the lowest distribution volumes (V_T) of all brain regions (*33*), same was true in our study. Nevertheless, the binding of [^{18}F]CPFPX can be displaced in the cerebellum as has previously been reported (*33*). The decrease in V_T that we observed in cerebellum during hypoxia was not significant ($p = 0.1074$).

Hypoxia can also be seen as a therapeutic tool for various diseases due to its role in regulating hematological, metabolic, angiogenic and neural adaptations. In colorectal cancer, systemic hypoxia combined with exercise not only has beneficial prognostic effects but also improves the quality of life measured on the basis of sleep quality, subjective pain scale, mental fatigue, and motivation (*34*). Furthermore, exposure to intermittent hypoxia over a 24-week period can also be used preventively in older adults to positively influence body fat mass management, reduce inflammation parameter levels and improve blood biomarkers of bone remodeling (*35*). Combining hypoxia with resistance training induces hormonal fluctuations in calcium, phosphate, bicarbonate, cortisol, and growth hormone levels (*36*). While the precise physiological mechanisms behind these hormonal changes remain

uncertain, they appear to stem not from changes in the hypothalamic pituitary axis, but rather from exhaustion and lactate accumulation during exercise (*37*).

Ischemic strokes, which account for 87 % of all stroke cases (*38*), are characterized by localized deficiency of oxygen in the brain tissue. A_1AR and its partial agonists have been shown to significantly aid in the recovery of synaptic transmission following ischemic stroke, highlighting their therapeutic potential (*39*). A_1AR additionally plays a central role in the activation, proliferation and inflammation of glial cells after experimental stroke (*40*). This underlines the many possible applications of future drugs and methods. Further research is required to better elucidate this mechanism for stroke treatment, which has a large socioeconomic impact in Europe by costing approximately 60 billion Euros per year (*41*). These costs are likely to rise due to an ageing population, therefore a more effective potential treatment is crucial in the future.

4.3 Heart rate and vital parameters

Heart rate increased by 19 % ($p = 0.0039$) under hypoxic conditions. Arterializing the blood of the left arm with a heating mat further ensured reliable measurements by making the SpO_2-sensors less error-prone. Because we were particularly dependent on the reliability of the measurement due to the study design and the planned drop in peripheral blood oxygen saturation to just above 70 %, we additionally used the Siemens internal oxygen clip on the other arm. Together with the ECG data, this provided three sources for the pulse data and two for peripheral oxygen saturation. The course of the SpO_2 and the small error bars in figure 8 also show how well controlled the hypoxia was in our study. This underlines the precision and reliability of the measurement results. We measured heart rate continuously and saw an increase during acute hypoxia as previously reported (*6*). Beyond this, we were again able to observe the course and speed of the heart's adaptation to acute hypoxia due to the high temporal resolution of data acquisition. Given the lower tolerance to ischemia and hypoxia with increasing age (*42*), this factor served as an additional reason for including younger participants, aged 19 to 46, in our study.

With the onset of hypoxia, heart rate increased rapidly within two min. This again is quicker than the change of A_1AR availability and comparable to the speed of change in brain perfusion. Even before the peripheral oxygen saturation dropped below 90 %, the body reacted to the onset of moderate hypoxia by a quick rise in heart rate simultaneous to the drop in inspired oxygen. With reaching the plateau after approximately five minutes of hypoxia, the heart rate remained elevated in all subjects. When oxygen supply was restored to 21 %, heart rate

consecutively decreased to the baseline level, even earlier than the peripheral oxygen saturation rebounded to baseline levels.

4.4 Psychomotor vigilance test

Compared to heart rate and perfusion, the relative hypoxia-induced decrease in reaction speed as well as the return to baseline were slower. This suggests that the system governing reaction speed is inherently more gradual. Considering that psychomotor vigilance is dependent on processing in different brain regions and is not subject to rapidly reacting physiological control loops like perfusion or heart rate (*43*), this slower adaptation to hypoxic conditions is logical. Therefore, we analyzed data from the latter half of the hypoxia phase for comparison with normoxia. Since a blood sample was drawn every ten minutes, a 3-minute version of the PVT was used, as it has been shown to achieve reliable results in other studies (*44, 58*).

Despite significant results in both PVT and A_1AR, a correlation of both values (see section 4.5) did not produce a statistically significant outcome. However, both the three-minute and the ten-minute PVT were validated for assessing sleep loss and alcohol-induced impairments in cognitive performance (*58*) and could therefore be too rudimentary for this context. A more complex assessment such as the d2 test, which evaluates intricate cognitive processes, might yield correlatable results (*45*).

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Supplemental Tables:

Supplemental Table 1: Correlation of time of day and V_T values (baseline and relative change)

Brain region	*Time of day and baseline*		*Time of day and rel change*	
	Pearson's r	*p value*	*Pearson's r*	*p value*
Frontal lobe	0.401	0.251	-0.31	0.384
Temporal lobe	0.425	0.221	-0.315	0.376
Occipital lobe	0.332	0.349	-0.322	0.364
Parietal lobe	0.349	0.324	-0.287	0.422
Striatum	0.295	0.408	-0.263	0.464
Insula	0.444	0.199	-0.331	0.35
Cingulate gyrus	0.426	0.22	-0.313	0.378

Supplemental Table 2: Correlation (r and p value) of each brain regions relative change of ASL and VT

Brain region	*Relative change of ASL and V_T*	
	Pearson's r	*p value*
Frontal lobe	0.018	0.962
Temporal lobe	0.163	0.652
Occipital lobe	-0.025	0.945
Parietal lobe	0.178	0.623
Striatum	0.106	0.77
Insula	0.028	0.939
Cingulate gyrus	-0.244	0.497