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Adenosine A_{2A} receptors in early ischemic vascular injury after subarachnoid hemorrhage

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Abstract

Object—The role of adenosine A_{2A} receptors (A_{2A}R) in the early vascular response after subarachnoid hemorrhage (SAH) is not known. In other forms of cerebral ischemia both activation and inhibition of A_{2A}R is reported to be beneficial. However, these studies mainly used pharmacological receptor modulation and most of the agents available exhibit low specificity. We used adenosine A_{2A} receptor knockout mice to study the role of A_{2A}R in the early vascular response to SAH.

Method—SAH was induced in the wild type (WT; C57BL/6) and A_{2A}R knockout mice (A_{2A}R-KO) by endovascular puncture. Cerebral blood flow (CBF), intracranial pressure (ICP) and blood pressure (BP) were recorded, cerebral perfusion pressure (CPP) was deduced. Animals were sacrificed at 1, 3 and 6 hours after SAH or sham surgery. Coronal brain sections were immunostained for collagen-IV; major protein of basal lamina. The internal diameter of major cerebral arteries and the area fraction of collagen-IV positive microvessels (<100µm) were determined.

Results—Initial ICP rise and CPP fall at SAH induction was similar but CBF fall was significantly smaller in A_{2A}R-KO as compared to WT cohorts. The internal diameter of major cerebral vessels decreased progressively after SAH. The extent of diameter reduction was significantly less in A_{2A}R-KO than in WT mice. Collagen-IV immunostaining decreased progressively after SAH. The decrease was significantly less in A_{2A}R-KO than in WT.

Conclusion—Our results demonstrate that global inactivation of A_{2A}R decreases the intensity of the early vascular response to SAH. Early inhibition of A_{2A}R after SAH might reduce cerebral injury.

Keywords

stroke; subarachnoid hemorrhage; mouse; microvessels; adenosine

Introduction

Cerebral injury after subarachnoid hemorrhage (SAH) is ischemic in nature. Two phases of cerebral ischemia are recognized; early (acute) ischemia which develops within the first 48 hours, and delayed ischemia with vasospasm, which develop 3–7 days later. Early ischemic brain injury after SAH accompanies small vessel injury, vasoconstriction, and reduced cerebral blood flow (CBF) and is the most important contributor to poor outcome after SAH.³¹

Adenosine is potent vasodilator which is implicated in CBF regulation especially in conditions, such as ischemia, which disturb supply and demand.^{25,37} The extracellular level of adenosine increases markedly during cerebral ischemia and is associated with vasodilation, decrease postsynaptic hyperpolarization, inhibition of free radical formation and platelet aggregation and platelet endothelial cell interaction. Adenosine receptors are G-protein coupled receptors and are found in at least four subtypes; A₁, A_{2A}, A_{2B}, and A₃. A₁ and A_{2A} receptors have been demonstrated to be mainly involved in adenosine mediated neuroprotection although role of A_{2B} receptors cannot be rolled out.⁶ A₁ and A_{2A} receptors have high and low affinity respectively for adenosine and when activated result, in most circumstances, in opposing functions. Interaction/cross talk between A₁ and A_{2A} receptors is established and lead to A_{2A} induced desensitization and down regulation of A₁ receptors and its response.^{11,19} In addition adenosine receptors also make functionally active dimers with each other (e.g. A₁/A_{2A} heteromers) or with other neurotransmitter receptors (e.g. A_{2A}/D₂ heteromers).¹² Thus, the capacity of adenosine to activate receptors with opposing functions allows it to serve as an upstream regulator that fine-tunes and integrates excitatory and inhibitory functions in the CNS.

Adenosine_{2A} receptors are located on smooth muscle of cerebral vasculature and are considered prime mediators of adenosine associated vasodilation.²¹ In addition, A_{2A} receptors agonists in endotoxemia and sepsis decrease inflammation and increase survival.^{23,36} Studies show that adenosine mediated vasodilation is impaired 48 hours after SAH⁸ and early administration of A_{2A} agonist prevents development of delayed cerebral vasospasm.¹⁸ However, if A_{2A} receptor plays a role during the early pathophysiology of SAH is not determined and studies on the role of A_{2A} receptor in other forms of ischemic brain injury have not reached a consensus.¹⁰ Using mainly pharmacological modulation, many investigators report activation and equally many inhibition of A_{2A} receptors to be beneficial against ischemic brain injury. Differences in the route of administration of drugs as well as the low specificity of A_{2A} receptor agonist and antagonists may underlie this controversy.^{10,24}

We here address this problem by comparing the effects of SAH on wild type and adenosine A_{2A}R-knockout (KO) mice.⁹ SAH was induced via endovascular puncture, an adaptation of the existing rat model.⁴ Intracranial pressure, cerebral blood flow and blood pressure responded to SAH identically in the two populations. The internal diameter of major cerebral vessels and area fraction of collagen IV in parenchymal vessels decreased after SAH in both WT and A_{2A}R-KO mice. However, the decrease was significantly less and developed at slower pace in A_{2A}R-KO. Our results indicate that the absence of functional adenosine A_{2A} receptors attenuates the intensity and slows the development of early vascular injury after SAH.

Materials and Methods

All experimental procedures and protocols used were approved by the Animal Care Committee of the Mount Sinai Medical Center. Wild-type and A_{2A} receptor C57Bl/6 mice (20–25gm) were used. The A_{2A} receptor knockout mice were generated by Chen et al.⁹ Homozygous A_{2A} knockout mice and C57Bl/6 wild-type mice were generated by homozygous interbreeding using the breeding pairs from the University of Virginia (Joel Linden). Since this breeding

strategy bypasses the need for genotyping by polymerase chain reaction or Southern analyses, the genotype of this breeding was confirmed by a functional test by using the A_{2A} receptor agonist CGS 21680 for hypotension response (not shown). In addition, breeding was limited to 3–5 generations in order to limit the potential confounding effect of spontaneous regression resulting from the interbreeding.

Endovascular mouse model of SAH

SAH was induced using an adaptation of the rat endovascular model of SAH.⁴ Mice were anesthetized with ketamine-xylazine (80mg/Kg+10mg/Kg; IP), transorally intubated, ventilated, and maintained on inspired isoflurane (1% to 2% in oxygen-supplemented room air). Mice were placed on a homeothermic blanket to maintain body temperature at 37°C and positioned in a stereotactic frame. The femoral artery was cannulated for blood gas and blood pressure monitoring. For ICP measurement, the atlanto-occipital membrane was exposed and cannulated, and the cannula was affixed with methymethacrylate cement to a stainless steel screw, implanted in the occipital bone. CBF was measured by laser-Doppler flowmetry by placing 0.8mm diameter needle probes (Vasamedics, Inc., St. Paul, MN, USA) directly over the skull away from large pial vessels in the distribution of the middle cerebral artery. This variation of our original SAH model is adopted to minimize injury.

SAH was induced by advancing a 5'0 polydioxanone monofilament retrograde through the ligated right external carotid artery and distally through the internal carotid artery (ICA) until the suture perforated the intracranial ICA bifurcation. This event was confirmed by a rapid rise in ICP and bilateral fall in CBF.⁴ Shams that received same surgical procedure as SAH animals expect for intracranial ICA perforation were used as controls. Mice were sacrificed at 1, 3 or 6 hours after SAH or sham surgery (n=4–5 per group per time point). SAH mortality in this study was 35%.

Characterization of the mouse endovascular model of SAH

Physiological measurements: To determine if mouse SAH mimics the human SAH, physiological changes upon SAH induction were recorded. These real time recordings included blood pressure (BP), intracranial pressure (ICP) and cerebral blood flow (CBF, ipsilateral and contralateral) recording (PolyView software; Grass Instruments; MS, USA) starting 20 minutes prior to 10 or 60 minutes after SAH induction. Cerebral perfusion pressure (CPP) was calculated by subtracting ICP from BP. CBF data were normalized to the baseline value averaged over 20 minutes prior to SAH, and expressed as a percentage of baselines. The variations in the peak ICP and CBF nadir values upon SAH induction were used to evaluate the consistency and reproducibility of the model.

Histological measurements, Brain preparation: These measurements studied the affect of SAH on major vessels and on parenchymal vessels. Briefly, animals were sacrificed by perfusion with chilled saline followed by 1% paraformaldehyde (PAF). Brains were removed with the meninges and the circle of Willis intact, embedded in Tissue-Tek OCT compound (Miles, Elkhart, IN), and frozen in 2-methylbutane cooled in dry ice. Sections located at bregma +0.0 to 0.38 mm and +0.38 to 0.74 mm were used. (www.mlb.org)

Collagen-IV Immunofluorescence

Collagen IV (constitutes up to 90% of the total protein of the basal lamina and confers structural integrity to the vessel wall) immunostaining was used to study major and parenchymal vessels. Collagen IV is Sections (8µm) were incubated overnight at 4°C with monoclonal anti-collagen IV goat anti Collagen-IV (Southern Biotech., USA), and then incubated with species-specific donkey anti-goat FITC conjugated secondary antibody (Jackson Immuno., USA) for overnight at 4°C. Finally, sections were washed and coverslipped.

Morphometry—Analysis was performed by an observer blinded to specimen identity. Quantitative analysis was performed on widefield images obtained under constant illumination and exposure settings using a 20x objective lens (field area $8 \times 10^4 \mu\text{m}^2$, Leica DM 6000 and DFC 350 FX camera).

Major vessel analysis: Brains were removed, preserving the meninges and the circle of Willis at the skull base and 8 μm coronal sections were immunostained for collagen IV. Collagen-IV immunostained major vessels were photographed and analyzed for wall thickness and internal and external circumference (IPLab, Scanalytic Inc, USA). Collagen-IV immunostained major vessels were photographed and analyzed for wall thickness and internal and external circumference as described previously (5,32). Briefly, since postfixation distortion of vessel wall shape can alter the measurement of the diameter or radius but does not affect the measurement of circumference. Hence, circumference was measured and diameter was calculated using the relationship circumference/ π . Although all brains were coronally sectioned in a rigid template, a separate potential influence on the measurement of circumference could be minor variations in the angle of histological sectioning. Therefore, in addition to measurement of the internal circumference, the thickness of the blood vessel wall was determined. Thickness was calculated as the average of 4 measurements made at 12, 3, 6, and 9 o'clock in order to minimize the effects of slice angle. Vessel measurements were made for internal carotid artery (ICA), proximal anterior cerebral artery (A1 segment) at bregma +0.0 to 0.38 mm.

Parenchymal vessels: Parenchymal vessels in four brain regions (basal and frontal cerebral cortex, striatum, and hippocampus), separated in right and left hemispheres, were analyzed. 2–3 fields per region per hemisphere were analyzed using IPLab software. Vascular profiles were isolated by intensity threshold segmentation and the number, average area and the area fractions of collagen-IV immunofluorescent vascular profiles were determined. The area fraction, a standard stereological measure, estimates the total amount of a partition as a fraction of total sample volume. The area fraction in this study estimates the total amount of collagen-IV positive tissue as fraction of tissue volume.

Statistics: Each parameter was analyzed by two-way ANOVA (StatView, SAS institute Inc. USA) followed, where appropriate, by Fisher's PLSD post-hoc t-tests.

Results

This study was divided in two parts; the first part developed and characterized the mouse endovascular model of SAH and compared it with the existing rat SAH model. The second part of study used the mouse endovascular to address the question; does adenosine A2 receptor has a role in early vascular injury after SAH?

Part -One

The mouse endovascular model of SAH—The rat endovascular model of SAH was adapted to mouse.

Physiology: Baseline ICP was $10 \pm 0.8 \text{ mmHg}$ (mean $\pm$ sem). At SAH, ICP rose immediately to $61 \pm 4 \text{ mmHg}$, thereafter declined slowly and by 60 minutes had reached $36 \pm 3 \text{ mmHg}$. (Figure-1, A) Baseline BP was $84 \pm 3 \text{ mmHg}$. BP increased slightly to 90 mmHg at SAH induction and was $78 \pm 3 \text{ mmHg}$ at 60 minutes. It should be noted that in mouse the upper and lower limit of BP for CBF autoregulation are 110 and 40 mmHg .³ Baseline CPP was $75 \pm 3 \text{ mmHg}$. Following SAH induction, CPP fell to $36 \pm 6 \text{ mmHg}$ (48% of the basal values) and remained near this value at 60 minutes. (Figure-1, C) CBF fell to $10 \pm 2\%$ of baseline at SAH

and had recovered to 36 ± 5 % of baseline 60 minutes later. (Figure-1, D) Please note that in addition to mouse, Figure 1 shows retrospective data of early physiological changes in rat after SAH. This figure is important for a comparison of rat and mouse SAH model; discussed in detail in discussion section.

Histological measurements: Internal carotid artery (ICA) and proximal anterior cerebral artery (A1 segment) displayed the similar temporal behavior to SAH; their data were pooled for analysis.

Internal circumference of sham operated WT animals sacrificed at 1 or 3 hours after surgery remained unchanged but had decreased 15% at 6 hours (Figure-3A, $P=0.044$). In contrast, a significant time dependent reduction of internal circumference of WT mice occurred after SAH (Figure 3B, $F=38.6$, $P<0.001$). Internal circumference decreased to 51% at 1 hour and recovered thereafter, reaching approximately 70% of time matched sham cohort 6 hours after SAH. Previously, we have found similar reductions in internal circumference of major vessels (ICA: 51%, A2 segment: 52%) in rat model one hour after SAH.⁵

Wall thickness of ICA and A1 segment of anterior cerebral artery increased significantly after SAH as compared to sham -operated animals ($F=10.3$, $P=0.0017$; data not shown). At 1 hour after SAH wall thickness was 149 % and decreased there after reaching 111% of time matched sham at 6 hours.

Part-2

A_{2A}R-knockout mice—The initial peak ICP and minimum CPP values after SAH were similar in the knockouts and in WT animals (figure-2). However, from thereon small difference in ICP values at all times was observed, mean ICP was lower in knockouts than in WT mice, however, this difference reached significance only at 20 minutes. Similarly, CPP values at 10 min and beyond were significantly different between the two groups, with the knockouts showing values nearer to baseline than the WT, however, this difference reached significance only at 60 minutes. CBF fell to a significantly lower value in knockouts, however exhibited a similar trend of recovery as for ICP and CPP. BP appeared to be unaffected by the knockout.

Major cerebral vessels

Internal circumference of major cerebral vessels in sham operated A_{2A}R-KO sacrificed at 3 hours was similar to their time matched WT sham cohorts. After SAH a significant time dependent reduction in internal circumference of major cerebral vessels was found in A_{2A}R-KO mice after SAH (Figure 3B, $F=11.3$, $P=0.001$). Internal circumference decreased to 80% at 1hr after SAH, remained near this value at 3 hours, and then decreased further, reaching approximately 60% of the sham values at 6 hours post SAH.

Compared to WT mice the degree of reduction in internal circumference of major cerebral arteries after SAH was significantly smaller in A_{2A}R-KO ($F=8.7$, $P=0.004$). This difference was significant at 1hour ($F=22$, $P=0.0001$), and a 3 hours ($F=5.09$; $P=0.03$). At 6 hours after SAH no difference in internal circumference of major cerebral arteries after SAH was found ($F=0.48$, $P=0.49$; figure 3B).

Wall thickness—Compared to sham-operated A_{2A}R-KO mice the wall thickness of major cerebral arteries increased significantly after SAH as ($F=4.5$, $P=0.03$). At 1hr, wall thickness increased to 141 % and decreased thereafter reaching a value not significantly different then sham (91 ± 4.7 %) at 6 hours after SAH. Increase in the wall thickness of major cerebral artery in KO was similar to that of WT at all time points after SAH ($F=0.001$, $P=0.9$; data not shown).

Collagen-IV immunofluorescence

Wild type mice—As reported previously for rats, in sham operated WT mice collagen-IV immunostaining clearly delineated both large vessels and the microvasculature. After SAH, parenchymal vessels appeared as interrupted sequences of segments instead of continuous profiles (Figure 4A). This staining pattern was present across the two hemispheres and in all brain regions; hence for quantification all measurements were pooled and shown as percent sham. In WT mice, collagen-IV immunostaining was decreased to 90% of normal area at 1 hour survival, the first time point assayed. Collagen-IV area decreased further to 70% of normal at 6 hours survival.(Figure 4B)

Adenosine A_{2A} receptor knockout mice—In contrast to the early reduction in collagen-IV immunostaining in the WT mice, the area fraction of collagen-IV immunostaining in the KO remained at normal levels at 1 and 3 hours survival. Immunostaining was reduced at 6 hours survival to 80 % of normal, a value significantly greater than the 6 hour WT value. ANOVA compared to WT ($F= 25.1$, $P<0.001$), post hoc test; $P<0.05$ at all time points examined. No hemispheric or brain regional difference in collagen IV staining in sham or SAH animals was observed.

These data indicate that the A_{2A} receptor knockout reduces microvessel degradation after SAH. (Figure 4A and B)

Discussion

This study adapted the rat endovascular model of SAH to mouse and investigated the role of adenosine A_{2A} receptor in ischemic vascular injury after SAH. In WT mice, temporal changes in wall thickness and internal diameter of major cerebral vessels and in microvascular collagen-IV were similar to those we have found in the rat.^{5,34} However, in A_{2A} receptor knockout mice these changes were reduced in intensity.

Existing Mouse models of SAH

Two variations of mouse experimental SAH models exist; endovascular puncture models^{15,27} and injection models (injection of autologous blood into the subarachnoid space)^{17,20}. Neither model record early physiological changes after SAH. Physiological data are important in interpreting the results of SAH experiments for a number of reasons: 1. Control of blood pressure and blood gases are required to assure that ischemic and other cerebral changes are due to the hemorrhage itself rather than systemic causes. 2. We and others have demonstrated that early alteration of CBF is one of the critical determinants of outcome after SAH^{5,29}. CBF is highly correlated with ischemic glutamate release during the last 60 minutes after SAH and predicts 24 hr survival⁵. CBF reduction can be attenuated pharmacologically to reduce glutamate release³². 3. Increased ICP at the time of SAH registers the event. When the rise in ICP is not confirmed there is no way of knowing if an SAH occurred when the animal is sacrificed at later time points, particularly in experiments that do not utilize histological analysis. The degree of elevation of ICP provides information regarding the intensity of the bleed³⁰, which permits stratification according to SAH intensity⁵. For these reasons physiological measurements in a mouse model of SAH provides an important tool in understanding SAH pathophysiology

The mouse endovascular SAH model: physiological changes and comparison with the rat

The value of ICP peak in the present endovascular mouse SAH model falls into moderately severe in the rat.⁵ A comparison of early physiological changes between mouse and rats that received SAH of similar intensity induced via similar method (Figure-1) indicates no difference in CBF and BP during the first 60 minutes, however, a quite different pattern of ICP decline

after reaching a peak. The pattern of decline ICP is important as it affects CPP. Two patterns of ICP declines are noted in humans, dogs, and baboons: 1) a fast decline (within 5 minutes) to a plateau at a value near the basal values and 2) a slow decline followed by a plateau at a value higher than the basal value.^{2,16} We have previously found the first pattern of ICP decay in the rat endovascular model of SAH and now document the second pattern of ICP decay pattern in the mouse. It is important to note that, in the first pattern, as ICP returns to near basal values, so does the CPP; however, in the second pattern, depending upon the value of ICP plateau, CPP remains reduced. As a consequence of the ICP pattern, CPP at 60 minutes after SAH recovers to approximately 80% of baseline in rat, and only 48% of baseline in mouse. Substantial CPP reduction in the mouse after SAH would be an additional factor contributing in alterations in cerebral microcirculation. The persistent elevation of ICP (pattern II) has been attributed to the occurrence of cisternal hematoma that would make recirculation impossible and create a clinical picture of brain death. This ICP pattern is considered responsible of the wide distribution of the necrotic foci in human SAH seen at autopsy that could not be ascribed to cerebral vasospasm.²

The mouse endovascular SAH model; major and parenchymal vessel injury

We have found changes in major and parenchymal vessels in WT mice that are similar to those previously documented in the rat after SAH.^{5,34} In this study changes in internal diameter and wall thickness of major cerebral vessels occur as early as one hour after SAH and have begun to recover to the basal level by 6 hours, the last time interval examined in this study. Previous studies show similar changes in major cerebral vessels in rat after SAH.⁵ The loss of the major basal lamina protein, collagen-IV, had already begun at one hour after SAH and continued at 6 hours. We have previously described a similar temporal profile of collagen-IV loss in rat after SAH.³⁴ These results strengthen our previous finding and establish cross specie similarity in early effects of SAH on cerebral vessels.

Adenosine A_{2A} receptors knock out mice and major and parenchymal vessel injury after SAH

Our results indicate that A_{2A}R-KO animals are more resistant to vascular changes and injury after SAH than the WT mice. Although constriction of major cerebral vessels and reduction in collagen IV area fraction of parenchymal vessels occurred in both A_{2A}R-KO and WT mice, the temporal profile and intensity of their development was different. For example: at 1 and 3 hours after SAH internal diameter of major vessels had reduced by 20 % in A_{2A}R-KO and by 50–60% in WT cohorts. Similarly, the area fraction of collagen IV decreased only at 6 hours after SAH in A_{2A}R-KO but had already reduced at one hour in the wild types. This result indicates that absence of functional A_{2A}R attenuates brain vascular injury during the early hours of SAH.

The contribution of A_{2A} receptors in other forms of cerebral ischemic injury is studied with mixed results. Some investigators find A_{2A} receptor activation beneficial^{1,35} and others harmful to the ischemic brain.^{7,13,14,22,28} This may be due to the lack of selectivity of the pharmacological agents used. Most A_{2A} receptor antagonists and agonist act on all adenosine receptor subtype with different affinities.^{24,26} Although, affinity based discrimination are achievable with *in vitro* studies, selectivity is more difficult with *in vivo* studies due to the high dose requirements. The use of genetically manipulated animals is a research strategy which avoids pharmacological pitfalls.

Using a middle cerebral artery model of cerebral ischemia Chen et al., have demonstrated that global A_{2A} receptor inactivation reduces infarct volume and improves neurological behavioral deficit score.⁹ More recently, the same group generated chimeric mice by selectively inactivating or reconstituting A_{2A} receptors into knockout or wild-type littermates and assessed response to cerebral ischemic injury.³⁸ They found that selective inactivation of A_{2A} receptors

attenuates infarct volumes and ischemia-induced expression of several proinflammatory cytokines and their selective reconstitution reinstates ischemic brain injury. The results from our study indicate that protection against the early vascular changes is yet another mechanism of attenuated cerebral injury in the absence of functional A_{2A} receptors. Lastly, Miekisiak et al have demonstrated significant attenuation of CBF during transient (30 seconds) of hypoxia in A_{2A}R-KO as compared to WT animals.²¹

We speculate that A_{2A}R-KO mice are resistant to post-SAH change in vessel diameter and basal lamina because the absence of functional A_{2A} receptors allows expression of vaso-protective mechanisms. One mechanism may be the lack of desensitization of A₁ receptors by A_{2A} receptor in A_{2A}R-KO mice. A number of studies demonstrate that A₁ receptors are desensitized and down regulate by A_{2A} receptors and their response^{11,19}, and hence selective inhibition of A_{2A} receptors would attenuate ischemic injury.¹⁴

Another mechanism may involve A_{2B} receptors. A_{2B} receptors are present in small number on vascular smooth muscle and have vasodilator properties. In the absence of functional A_{2A}R in our KO animals, adenosine concentrations may be elevated. The increased concentrations of adenosine after SAH in the A_{2A}R-KO animals would lead to activation of the less sensitive A_{2B} receptors. Alternatively, A_{2B} activation might become more pronounced in the absence of functional A_{2A} receptors. Either mechanism, increased adenosine concentrations in KO mice or increased activation of A_{2B} receptors, could account for the reduced collagen loss after SAH in A_{2A}R KO mice. We have previously demonstrated that matrix metalloproteinase-9 (a collagenase; MMP-9) is involved in collagen-IV destruction after SAH.^{33,34} A recent study demonstrates that during hypoxia activation of A_{2B} receptors lead to inhibition of MMP-9 production in monocyte-derived dendritic cells required for their transfer across extracellular matrix.³⁹

Similarity in physiology but not in vascular effects of SAH in WT and A_{2A}R mice

It should be noted that in this study the intensity of alterations of major and parenchyma vessel after SAH varied between the two genotypes while only small difference in the physiological parameters were found. More specifically, the peak rise and 60 minute decline in ICP, 60 minute recovery in CBF, and initial fall in CPP were comparable among the WT and A_{2A}R-KO mice indicating similar intensity of injury but the degree of reduction in major vessels diameter and the denudation of collagen-IV of parenchymal vessels basal lamina varied. This disparity between physiology and vascular response indicates contribution of additional mechanisms in vascular injury after SAH. Moreover, the similarity in the magnitude of CBF reduction, but not in the diameter of major vessels between the two mouse strains indicates that mere reduction in major vessel diameter can not explain circulation deficits after SAH. Other contributing mechanisms may include; reduced cerebral nitric oxide level, aggregation of platelets in microvessels, release and activation of vascular collagenases such as matrix metalloproteinases 9 (MMP-9), and etc.³¹

Conclusion

Absence of functional A_{2A}R decreases ICP and increases CPP, reduces the constriction of major vessels, and reduces the loss of microvascular collagen-IV after SAH. These results suggest that adenosine signaling plays a role in vascular dynamics and microvascular injury in the period immediately following SAH and, further, that early inhibition of adenosine A_{2A} receptors after SAH may prove beneficial.

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Figure-1. A comparison of physiological changes after rat and mouse SAH induced via endovascular model

SAH rats that had similar ICP values as WT SAH mouse were used for this comparison. (Retrospective data from rat endovascular model) Note the difference in the pattern of ICP decline between the two species after SAH (A; see text for explanation). Baseline BP (B) was slightly lower in mouse and remained unchanged (as in rat) after SAH (B). Initial fall in CPP at SAH was the same but recovery was lower in mouse (C). CBF fall at SAH and recovery was the same between the two species (D).

Figure-2. A comparison of physiological changes after SAH in WT and A_{2A}R-KO

Small but significant differences in physiological parameter after SAH were found. ICP rose to the similar value at SAH but decline to a smaller value at 10–20 minutes in A_{2A}R-KO (A). At 60 minutes after SAH ICP plateau to the same value in WT and A_{2A}R-KO mice. No difference in BP values before or after SAH occurred in WT and KO mice (B). Initial CPP fall was similar but a trend towards higher recovery thereafter was seen in A_{2A}R-KO (C). CBF fell to a significantly smaller value in KO but recovered to the similar value at 60 minutes after SAH. * Significantly different than rat SAH at P<0.05.

Figure-3. Internal circumference of Major cerebral vessels in WT and A_{2A}R-KO after SAH

A: Representative images of collagen-IV immunostained ICA from WT mice sacrificed 1 or 6 hours after sham or SAH surgeries. Internal circumference and wall thickness of 1hr-sham: 368μm and 41μm, 1hr-SAH: 191 and 66μm, 6hr-Sham: 305, and 39μm and, 6hr-SAH: 219 and 46μm. Scale bar: 50μm. **B:** Mice were sacrificed 1–6 hours after SAH or sham surgery. Internal circumference of WT shams was similar at 1 and 3 hours but decreased by 15% at 6 hours. Internal circumference A_{2A}R-KO shams was similar to their time matched WT sham cohorts. In contrast, a significant time dependent reduction in internal circumference of WT and A_{2A}R KO mice occurred after SAH. The magnitude of internal circumference reduction at 1 and 3hrs after SAH was significantly more in WT than in A_{2A}R-KO. * Significantly different than sham at P<0.05. ! Significantly different than A_{2A}R-KO at P<0.05.

Figure-4. Collagen-IV immunostaining of parenchymal microvessels is reduced after SAH

A: Representative images of collagen-IV immunostaining of microvessels from striatum of WT and A_{2A}R-KO mice sacrificed 1 hr after SAH. Note more complete and longer collagen-IV stained vascular profiles in A_{2A}R-KO mice as compared to the time matched WT cohorts.

B: Accumulative graph of the area fraction % respective sham of collagen-IV positive vascular profiles in WT and A_{2A}R-KO mice sacrificed 1 to 6 hours after SAH. Note the delayed decrease in collagen-IV immunostaining in A_{2A}R-KO as compared to WT mice after SAH. *

Significantly different than sham operated animals at $P < 0.05$, ! Significantly different than the time matched A_{2A}R-KO cohort at $P < 0.05$.