Review

Localization of the hydrogen sulfide and oxytocin systems at the depth of the sulci in a porcine model of acute subdural hematoma

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Abstract

In the porcine model discussed in this review, the acute subdural hematoma was induced by subdural injection of autologous blood over the left parietal cortex, which led to a transient elevation of the intracerebral pressure, measured by bilateral neuromonitoring. The hematoma-induced brain injury was associated with albumin extravasation, oxidative stress, reactive astrogliosis and microglial activation in the ipsilateral hemisphere. Further proteins and injury markers were validated to be used for immunohistochemistry of porcine brain tissue. The cerebral expression patterns of oxytocin, oxytocin receptor, cystathionine-γ-lyase and cystathionine-β-synthase were particularly interesting: these four proteins all co-localized at the base of the sulci, where pressure-induced brain injury elicits maximum stress. In this context, the pig is a very relevant translational model in contrast to the rodent brain. The structure of the porcine brain is very similar to the human: the presence of gyri and sulci (gyrencephalic brain), white matter to grey matter proportion and tentorium cerebelli. Thus, pressure-induced injury in the porcine brain, unlike in the rodent brain, is reflective of the human pathophysiology.

Key Words: animal modeling; brain edema; cystathionine-β-synthase; cystathionine-γ-lyase; gyrencephalic brain; immunohistochemistry; intensive care unit; large animal model; neuromonitoring; oxytocin receptor

Introduction

This invited review discusses the potential significance of our recent published findings in a long term, resuscitated porcine pre-clinical model of acute subdural hematoma (ASDH) induced brain injury with continuous bilateral multimodal brain monitoring, brain targeted intensive care (Datzmann et al., 2021) and the subsequent cerebral immunohistochemical characterization of the injury caused by the ASDH (Denoix et al., 2020b). We found that i) ASDH was associated with morphological damage, which was only related to the hematoma, and ii) local brain injury was not reflected in the systemic markers evaluated, most likely due to the neuro-intensive care, which attenuated impairment of tissue energy metabolism and subsequent neurological dysfunction (Datzmann et al., 2021). Furthermore, we identified and validated novel protein targets for the characterization of cerebral injury, that can be used in the pig brain to further elucidate the pathophysiology of ASDH and the efficacy of potential therapeutic approaches (Denoix et al., 2020b). Specifically, biological markers of two systems relevant for fluid regulation in the brain, namely the hydrogen sulfide- and oxytocin systems, have been identified to co-localize at the base of the sulci, where pressure-induced injury, and thus potentially edema formation, is most pronounced in the gyrencephalic brain. Many of the pathophysiological changes in response to ASDH observed in this model were shown to be comparable between humans and pigs, probably due to the shared anatomical characteristics as well as intensive care management (Denoix et al., 2020b; Datzmann et al., 2021).

Relevance of Acute Subdural Hematoma Research

In brief, ASDH is a complication in 5–25% of traumatic brain injury (TBI) patients, as a consequence of tearing of bridging veins. In 2016, there were more than 27 million cases of TBI worldwide, as summarized in a systematic analysis for the Global Burden of Disease Study (GBD 2016 Traumatic Brain Injury and Spinal Cord Injury Collaborators, 2019). Patients in the age group below 70 years more frequently undergo severe TBI than mild TBI, whereas the overall incidence of TBI increases in the elderly population (> 70 years) with older patients more frequently experiencing mild TBI (GBD 2016 Traumatic Brain Injury and Spinal Cord Injury Collaborators, 2019; Lee et al., 2018). These older patients in particular have a higher incidence of ASDH associated with head injuries caused by falls (Evans et al., 2018; Lee et al., 2018). TBI patients present with a high incidence of ASDH and have a risk

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for suffering from long-term consequences including cognitive deficits and post-traumatic stress disorder (Bryant, 2011; Bramlett and Dietrich, 2015; Mauri et al., 2017; Lee et al., 2018), independent of their age. The molecular mechanisms for these long-term impairments and how different levels of brain injury can impact the psychological response are largely unknown (Bryant, 2011).

Relevance of Anatomy for Translation/ Translational Relevance of Animal Models

One of the greatest challenges to translation is choosing the appropriate animal model, which reflects the human anatomy and pathophysiology of the disease or injury state being reproduced. Animal models of brain injury mostly use young and healthy rodents which are essential for the understanding of basic physiological mechanisms. It is reasonable to try to reduce inter-individual variation by choosing animals of the same sex, age and strain in order to establish reproducible and defined conditions and control for physiology. These models may provide valuable and unique insights into the specific pathophysiology of experimental questions and may even reveal mechanisms for novel therapeutic targets (McCook et al., 2012). Unfortunately, the use of young healthy rodents for pre-clinical studies, which are the most commonly used animals in models of TBI, have frequently failed to translate into relevant clinical therapies (Nyanzu et al., 2017). This failure to translate from the bench to the bedside is one of the problems with research conducted in naive animals, wherein often a dramatic benefit is observed that cannot be reproduced in the clinical study. In a systemic review of preclinical and clinical trials it was reported that the discordance was due, at least in part, to the failure in the preclinical trial to properly mimic the clinical disease (Perel et al., 2007; McCook et al., 2012). Rodent models have predominated in TBI research because of their low cost, less ethical issues, and minimal post-surgical maintenance. Ironically, they are poor candidates for translational studies for human clinical interventions due to large species differences in neuronal anatomy, physiological and behavioral factors which are not representative of the human (Morganti-Kossmann et al., 2010; Vink and Bullock, 2010; Teo et al., 2012; Nyanzu et al., 2017; Vink, 2018). An important aspect in modeling in translational TBI research is the surface anatomy of the brain: the lissencephalic nature of the rodent brain makes it a poor candidate for translation to humans who have gyrencephalic brains. The morphology of the gyrencephalic pig brain leads to both grey and white matter responses to injury, is closer to the human in cerebrovascular anatomy (location and distribution of surface vessels) as well as physiology, and the gyri influence the distribution of the delivery of force to structures at the depths of the brain: in particular the gyri affect the movement of the brain within the skull in response to impact injury and display much more morphological brain alterations than lissencephalic brains (Duhaime, 2006; Vink, 2018). In the lissencephalic brain "maximum mechanical stress" is experienced close to the surface and more evenly distributed, whereas in the gyrencephalic brain, as previously mentioned, the maximum stress is re-focused, away from the surface, to the depths at the base of the sulci as reported for humans (Barrio et al., 2015; McKee et al., 2015; Vink, 2018; Denoix et al., 2020b; **Figure 1**).

The distribution of white to grey matter is an important factor for translational neuro-trauma research. Not only is it relevant for morphology (Duhaime et al., 2006), but white matter is more prone to swelling and thus crucial to pressure induced injury (Vink, 2018; Muñoz-Maniega et al., 2019; Denoix et al., 2020b). Here again, the rodent proves to be a less appropriate choice for translational purposes: the rodent brain for instance is made up of 12–14% white matter in contrast to either the adult pig and human brain consisting of 60% white matter (Ahmad et al., 2015; Kinder et al., 2018; Denoix et al., 2020b; **Figure 1**). This may suggest that the pathophysiology of a traumatic injury in the lissencephalic rodent would be very different than in the gyrencephalic brain of either a human or a higher developed species (e.g., pig), which share anatomical and morphological features not only because of the gyri and sulci but also in the distribution of white matter. Another important feature, in which the pig is more similar to the human than rodents, is in the skull, not only in the bony cranial anatomy but the anatomy within the skull as well, and in the structure of the tentorium cerebelli. In humans and pigs both the skull and tentorium cerebelli are rigid in contrasts to rodents which only have a "vestigial connective tissue membrane" (Klintworth, 1968; Bull, 1969; Vink, 2018; Denoix et al., 2020b; **Figure 2**).

This latter discrepancy between rodents and humans assumes importance for neuro-trauma translational research in that the rigidity of the tentorium cerebelli, which separates the cerebrum from the cerebellum, is significant in confining the injury-induced edema formation and increase in intracranial pressure (ICP) following brain injury. In the rodent, the loose connective tissue that comprises the tentorium cerebelli allows for redistribution of pressure increases in the cerebrum to other brain areas, whereas the rigid structure in humans (and pigs) confines the increase of ICP to the cerebrum (Klintworth, 1968; Denoix et al., 2020b; **Figure 2**). Thus, the pig is a translationally relevant animal model for TBI research, as has been reviewed recently (Vink, 2018; Kinder et al., 2019). Therefore, we chose the pig, with a gyrencephalic human-like brain, to establish our ASDH model, in that it may improve our chances at clinical translation due to the more similar neuroanatomical structure (Datzmann et al., 2021). Furthermore, we instituted intensive care management, in that it is a standard procedure in human therapy and a common missing factor in animal models, which limits their translational relevance, especially, considering the clinical situation of TBI/ASDH patients (Duhaime 2006; Nyanzu et al., 2017; Datzmann et al., 2021).

Treatment of Acute Subdural Hematoma

The current therapeutic management of ASDH, following the "Guidelines for the Management of Severe Traumatic Brain Injury" (Carney et al., 2014); i.e. decompression, anesthesia and analgesia, neuro-monitoring (intracranial pressure, ICP; cerebral perfusion pressure, CPP), brain temperature and partial pressure of oxygen in brain tissue ($P_{bt}O_2$), hemodynamic monitoring, mechanical ventilation; requires the admission of the patient to the intensive care unit (ICU).

As emphasized above, ASDH management requires admission of the patient to the ICU and yet the lack of intensive care management is one of the most blatant omissions in rodent TBI models: in fact in three (Wojnarowicz, et al., 2017; Bodnar et al., 2019, systematic review; Skotak et al., 2019, comprehensive review) out of four (Nyanzu et al., 2017) recent reviews of rodent TBI models there is not even a mention of intensive care management. Only Nyanzu et al. (2017) pointed out the fact that "TBI models usually omit one or more critical and clinically essential pathophysiological feature": standard neuro-intensive monitoring and management: ICP monitoring, sedation, mechanical ventilation and cardiovascular support, and emphasizes that these must be incorporated into TBI modeling.

The ICU, in brief, allows for specialized medical care, monitoring and physiologic organ support of critically ill patients to sustain life in a life-threatening situation (Marshall et al., 2016). The late Daniel Traber (1999) pointed out many years ago that no critical care physician would accept drug efficacy data from studies of critical illness that did not include resuscitation, blood pressure or heart rate monitoring, yet
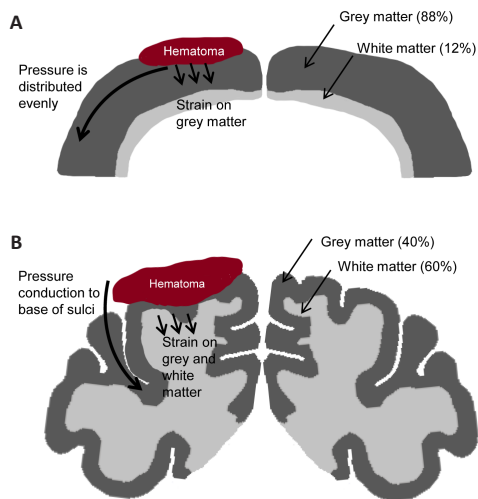
Review

Figure 1 | **Pressure distribution in the cerebral cortex after ASDH.**
(A) In the lissencephalic cerebral cortex, pressure increase in response to ASDH is distributed evenly. (B) In the gyrencephalic cerebral cortex, pressure increase in response to ASDH is conducted along the sulci, with maximum pressure at the base of the sulci. The size of the lissencephalic brain in comparison to the gyrencephalic brain is overestimated. ASDH: Acute subdural hematoma.

Figure 2 | **Schematic depiction of the brain within the skull.**
(A) In the rodent brain, the vestigial tentorium cerebelli can mitigate an elevation of intracranial pressure. (B) In the human (and porcine) brain, elevated intracranial pressure is confined to the cerebrum by the rigid tentorium cerebelli. The size of the rodent brain in comparison to the human brain is overestimated.

that is exactly what the failed rodent TBI studies did not do. What is important to understand is that the pathophysiology of an unresuscitated and unmonitored patient is essentially different from that of a resuscitated and monitored patient in an ICU. For those interested in implementing ICU management strategies in their rodent TBI models to improve their translational relevance there are examples of small rodent ICU´s discussed by Guillon et al. (2019). A chapter in the Methods in Molecular Biology Series (Merz et al., 2021), devoted to and entitled the Mouse Intensive Care Unit, MICU, includes a detailed description of the equipment required and how to perform the necessary procedures. A schematic picture of the MICU is shown in **Figure 3A**. In large animal models, the limitations for translation persist though they are not necessarily related to cerebral anatomy nor skull structure, as with the rodents, but due to lack of intensive care procedures (Van den Heuvel et al., 1999; Alessandri et al., 2003; Anderson et al., 2003; Malhotra et al., 2003; Timaru-Kast et al., 2008; Meissner et al., 2011; Friess et al., 2012; Armstead et al., 2017). Large animal models of neuro-trauma usually include mechanical ventilation and some level of resuscitation, are usually of short duration (2–12 hours) only, and do not often follow standard guidelines for intensive care parameters nor include neurological assessment (Nyanzu et al., 2017; DeWitt et al., 2018). A handful of pig models of ASDH using multimodal brain monitoring have been described for up to 12 hours but using only small injected blood volumes of 2–9 mL (Timaru-Kast et al., 2008; Meissner et al., 2011; Nielsen et al., 2011). Studies on ASDH in piglets have also described neurological deficits at different time points (Shaver et al., 1996), and of varying age (Durham and Duhaime, 2007), but these animals did not receive intensive care.

Integrating the guideline based intensive care therapy, we set out to establish a clinically relevant, resuscitated long-term (54 h) large (human sized) porcine model of ASDH to contribute to the understanding of ASDH pathophysiology and to be able to test novel therapeutic approaches (Datzmann et al., 2021). The model comprised bilateral multimodal brain monitoring (ICP, CPP, $P_{bt}O_2$, and brain temperature, see **Figure 3B**), neuro-intensive care, neurological assessment, repetitive blood sampling and postmortem tissue analysis (Datzmann et al., 2021).
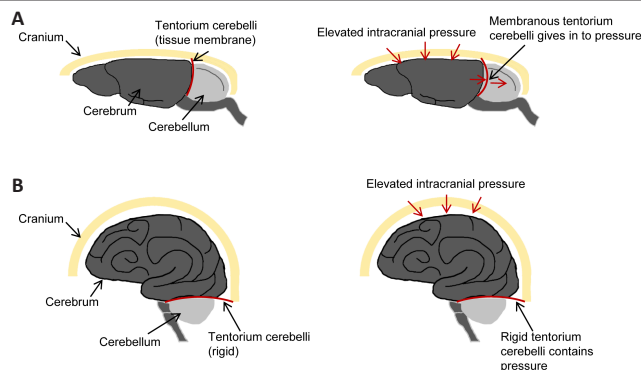
Pathophysiology of Acute Subdural Hematoma

ASDH primarily induces mechanical brain injury due to an increase in ICP as a consequence of subdural accumulation of blood (Hamahata and Nakagawa, 2020) and can lead to distortions, shifts and herniation of the brain parenchyma (Nyanzu et al., 2017). Vascular collapse, cerebral hypo-perfusion, inflammation and hypoxia might cause secondary brain injury and thus aggravate tissue damage (Hamahata and Nagakawa, 2020). The potential herniation of the brain parenchyma and ischemia are reasons to use neurointensive monitoring and management of both ICP and CCP in an effort to attenuate the neurodegeneration associated with the brain injury (Nyanzu et al., 2017). Intracranial hypertension has been shown to be strongly associated to the neurological deterioration after TBI (Nyanzu et al., 2017).

In our porcine model of ASDH, we found that the maintenance of CPP and $P_{bt}O_2$, in spite of the morphological damage, prevented severe impairment of tissue energy metabolism and, subsequently, major neurological dysfunction (Datzmann et al., 2021). The cerebrovascular dilation in response to elevated ICP might contribute to blood brain barrier (BBB) dysfunction, which can result in cerebral edema formation (Nemoto, 2006). Cerebral edema, in turn, can aggravate the ICP elevation, resulting in a vicious cycle (Nemoto, 2006). The molecular mechanisms of cerebral edema formation have been reviewed recently (Stokum et al., 2016).

Locally, cerebral fluid balance is regulated by the endothelial cells of the BBB: in the healthy brain, water is exchanged between the vasculature and interstitium without net water movements (Bering, 1952). During ASDH, inflammation and hypoxia induce tissue and cell damage, i.e. impaired cellular metabolism, oxidative stress, necrosis and apoptosis (Hossmann, 1999; Lacerte et al., 2020), which can impair endothelial function and result in an increase in endothelial permeability and albumin extravasation, i.e. barrier dysfunction (Engelhardt et al., 2014). In our ASDH model the peri-hematomal area, in contrast to the uninjured contralateral hemisphere, presented with increased markers of oxidative and nitrosative stress and extravasated albumin which indicated disruption of the BBB (**Figure 4**; Datzmann et al., 2021). Thus to better understand how the above mechanisms might be reflected in our ASDH model, we performed a more extensive analysis of the spatial expression patterns of proteins from the post-mortem tissue analysis which might be involved in edema formation, fluid regulation, BBB dysfunction, neuro-inflammation and oxidative stress (Denoix et al., 2020b). The markers of neuro-inflammation and complement activation displayed a pattern that corresponded
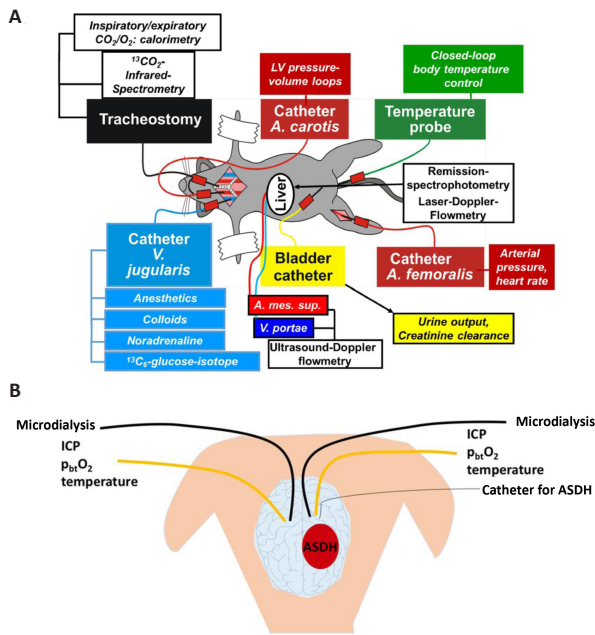
Figure 3 | Schematic depiction of animal intensive care unit concepts.
(A) The mouse intensive care unit (MICU) allows for monitoring and resuscitation of the animal during critical illness studies. A. mes. sup.: Superior mesenteric artery. Reprinted with permission from Guillon et al., 2019 (creative commons license, https://creativecommons.org/licenses/by/4.0/). (B) Additional multimodal brain monitoring in the acute subdural hematoma (ASDH) model in the pig intensive care unit (PICU) comprising the same ICU setup as the MICU. Reprinted with permission from Datzmann et al., 2021 (reprinted with permission from the American Association of Neurological Surgeons).

to what is reported for human TBI (Denoix et al., 2020b). Further proteins of interest were endogenous enzymes, cystathionine-γ-lyase (CSE) and cystathionine-β-synthase (CBS), producing the gaseous mediator hydrogen sulfide (H_2S) and the neuroendocrine peptide oxytocin (OT) and its receptor (OTR). The hydrogen sulfide and oxytocin systems are reported to interact with one another, in the hypothalamic supraoptic (SON) and paraventricular nuclei (PVN), in fluid homeostasis and cardiovascular regulation as well as in both physically and psychologically induced trauma, as recently reviewed (Denoix et al., 2020a). The hypothalamus is the central integrative structure regulating blood and body osmolality as well as fluid volume, and regulates blood pressure and heart rate in response to fluid shifts (Horn and Waldrop, 1997; Kinsman et al., 2017). Thus we were particularly interested in the role that H_2S and oxytocin systems may be playing in our model of ASDH induced neuro-trauma, which was characterized by BBB dysfunction and edema formation.

Furthermore, understanding the role of the H_2S and OT systems in the pathophysiology of ASDH might help to identify potential underlying molecular mechanisms of the long-term psychological response to TBI. Recent studies underscore the fact that physical and psychological trauma share physiological correlates (Auxemery, 2018; Denoix et al., 2020a), and both systems (H_2S and OT) are relevant mediators in models of both psychological and physical trauma, recently reviewed by Denoix et al. (2020a). H_2S and OT have parallel effects, converge on the same signaling cascades, and share the same signaling pathway, which further suggests their interaction. An acknowledged yet poorly understood consequence of TBI is the development of PTSD, associated with a rate of 14–77% of major depression disorder (Mauri et al., 2017). In the civilian population, PTSD is reported to be twice as frequent with mild TBI than in patients suffering physical injuries without TBI (Vasterling et al., 2018). In contrast, in the military population, the incidence of PTSD increases with the severity
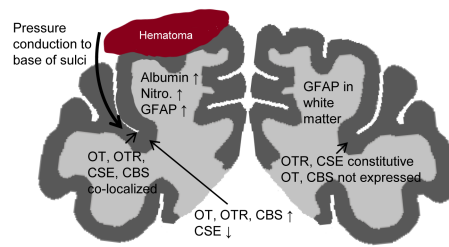

Figure 4 | Protein expression in the gyrencephalic cerebral cortex after ASDH.
Albumin extravasation, nitrotyrosine (Nitro.) formation and reactive astrogliosis were observed around the ASDH induction site (Datzmann et al., 2021). OT, OTR, CSE, CBS co-localized around the base of the sulci, where the maximum pressure-induced injury occurs (Denoix et al., 2020). In the parenchyma, OT, OTR and CBS expression were elevated in the injured compared to the contralateral hemisphere. In the contralateral hemisphere, OTR and CSE expression were constitutive (Denoix et al., 2020). GFAP expression was limited to the white matter, albumin extravasation and nitrotyrosine formation were not detected (Datzmann et al., 2021). ASDH: Acute subdural hematoma; CBS: cystathionine-β-synthase; CSE: cystathionine-γ-lyase; GFAP: glial fibrillary acidic protein; OT: oxytocin; OTR: oxytocin receptor.

of the TBI (Vasterling et al., 2018). PTSD is postulated to be associated with a dysregulated response to stress, and the neurobiological activation is thought to affect brain function and structure: amygdala, hippocampus, locus coeruleus, dorsal raphe nucleus and prefrontal cortex (Brenner, 2011). The observation of the H_2S and OT system being activated in the prefrontal cortex (Denoix et al., 2020b) assumes importance in that this is one of the very regions of the brain reported to be activated in PTSD (Brenner, 2011; Mauri et al., 2017).

Oxytocin as well as, more recently, H_2S have been reported to be involved in psychological disorders (Denoix et al., 2020a). In particular, OT administration has been evaluated in two PTSD clinical trials with limited but encouraging results (Frijling, 2017; Flanagan et al., 2018). In a very recent experimental single prolonged stress model of PTSD in rats, OT administration led to a change in phenotype from vulnerable to resilient and restored morphological changes in the prelimbic cortex and basolateral amygdala (Le Dorze et al., 2020). Thus, these novel findings in a clinically relevant porcine model depicting the interaction of the H_2S/CSE and OT systems in the pathophysiology of ASDH (Denoix et al., 2020b) may also hint to the biological mechanisms involved in PTSD. Furthermore, these findings not only provide potential for new therapeutic targets but also offer a small window into the mutual interplay of the body and psyche (Denoix et al., 2020a).

The Role of Hydrogen Sulfide in Vascular Protection and Barrier Dysfunction

In the context of fluid regulation, H_2S and OT have been reported to play a significant role: H_2S can stimulate hypothalamic OT release in response to fluid shifts (Coletti et al., 2015). Abe and Kimura (1996) were the first to identify H_2S as a neuromodulator. Furthermore, H_2S has anti-inflammatory effects and is important for oxygen sensing and vascular function. Given the fact, that CSE among the factors seems to be crucial for the integrity of the endothelial barrier (Stenzel et al., 2016), it is not surprising that protective effects of H_2S on the BBB after cardiac arrest and cerebral ischemia have been reported in the literature (Geng et al., 2014; Wang et al., 2014; Marutani et al., 2015; Li et al., 2018) and exogenous H_2S administration mediated vasculo-protection after cerebral ischemia (Wen et al., 2016). It should be noted however that the inhibition of endogenous H_2S synthesis also mediated an attenuation of BBB dysfunction in cerebral ischemia (Jiang et al., 2015),

suggesting a complex role for H_2S in BBB (dys)function.

The Role of Oxytocin in Vascular Protection and Fluid Regulation

OT works through binding to the G-protein coupled OTR. It has also been reported to be produced locally in the heart, where it exerts cardioprotective, vasculoprotective and vasodilatory effects (Gutkowska et al., 2014; Rabow et al., 2018). The cardioprotective effects of OT might act in concert with H_2S (Merz et al., 2018; Denoix et al., 2020a). In the brain, OT has been reported to be a regulator of cerebral blood flow, either by mediating vaso-constrictory effects on small cerebral resistance vessels or vasodilating effects on the large cerebral arteries (Stolz-Born et al., 1996).

Oxytocin and Hydrogen Sulfide Localization in the Central Nervous System

Due to the similar CSE and OTR organ expression in the pigs and humans, which were pursued in clinically relevant circulatory shock models including ICU management (McCook et al., 2014; Merz et al., 2017, 2020a), we analyzed the brain expression patterns of CSE and OTR after physical trauma. The localization, mapping and distribution of OTR and CSE in the porcine brain could help to better understand their interaction.

OTR and CSE were present in almost every brain section we investigated, especially arteries and microvasculature that were positive for OTR and CSE. Similar to what is described for the human, the cerebral vasculature in the porcine brain also expresses CSE (Lee et al., 2009). H_2S reportedly improves the integrity of the barrier and attenuates cerebral edema, and the reduction of CSE expression close to injury sites coincided with increased albumin extravasation and barrier dysfunction (Diwaker et al., 2007; Lee et al., 2009; Paul et al., 2014; Datzmann et al., 2021). Denoix et al. (2020b) reported that OTR and CSE were also expressed in the arteries and microvasculature, suggesting that they might play a role in BBB integrity. The presence of OT/OTR in the vasculature is in line with recent reports of OTR upregulation observed in humans, as an adaptive stress response in "vascular profiles" associated with perivascular swelling and around micro-infarcts (McKay et al., 2019). A novel finding in our porcine ASDH model was the expression pattern of OT, OTR, CSE and CBS (**Figure 4**): all four proteins were localized in the gyri. CSE was constitutively expressed in the parenchyma and neurons in the non-injured hemisphere but reduced around the ASDH induction site. In contrast, CBS was not detected in the neurons although highly expressed in the parenchyma in response to injury. The four proteins of interest, OT, OTR, CSE and CBS, could be detected co-localized at the base of the sulci, where pressure-induced brain injury elicits maximum stress, same as reported in the human (Brierley, 1977; Graham, 1977; Denoix et al., 2020b). This would not have been possible to detect in a rodent model with lissencephalic brain anatomy lacking gyri and sulci.

We have also confirmed the presence of OT and OTR in the porcine hypothalamus similarly to their localization in the human brain (Denoix et al., 2020a). Novel findings were OTR in the cerebellum and CSE expression in the hypothalamus. In the human cerebellum, CSE has also been identified in cerebellar purkinje cells (Diwaker and Ravindranath, 2007; Paul et al., 2014), which is in agreement with our findings in the pig. The results show either co-localization or reciprocal expression of CSE and OTR in the hypothalamus (Denoix et al., 2020a), and cerebellum (Denoix et al., 2019) respectively, suggesting that in different regions there may be different interaction patterns. Co-localization may indeed be reflective of the tissue specific situation when OT release is stimulated by H_2S as it is described for the hypothalamus during fluid

shifts (Coletti et al., 2015). In the cerebellum, both systems might rather have complementary modes of actions, so that when either one is absent, the other can compensate to rescue the brain from damage due to barrier dysfunction, inflammation, oxidative stress or hypoxia.

Conclusion

In conclusion, we have described a clinically relevant, long-term, resuscitated porcine model of ASDH-induced acute brain injury. Our most salient find was the localization of the endogenous enzymes of the H_2S and OT/R system in neurons, vasculature and parenchyma at the base of the sulci, where pressure induced injury leads to maximal stress in the gyrencephalic brain (**Figure 4**). In particular, CSE expression was constitutive in intact brain regions and reduced at the site of injury, which coincided with its reciprocal expression to nitrotyrosine formation, a marker of oxidative and nitrosative stress (**Figure 4**) (Datzmann et al., 2021; Denoix et al., 2020b). OTR and the H_2S-producing enzymes were detected in the parenchyma, a common site of injury in ASDH (Kotwica and Brzezinski, 1993), which has not been reported before (Denoix et al., 2020b). Both systems are involved in cerebral and vascular fluid regulation (Coletti et al., 2015; Stenzel et al., 2016; Denoix et al., 2020a), ASDH-induced brain edema formation, and associated with psychological disorders (Denoix et al., 2020a). In particular, PTSD and other psychological disorders have been associated with a dysregulation in the pre-frontal cortex (Brenner, 2011; Mauri et al., 2017), which is the specific region, where the H_2S and OT/R systems were localized. Although there is no direct evidence, these findings are indicating potentially relevant biological mechanisms of ASDH-induced PTSD. Finally, we observed the same spatial relation to injury known for humans in our pig model, suggesting it may be an appropriate model to study human brain injury and to test the efficacy of novel therapeutic approaches.

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