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An insulin based model to explain changes and interactions in human breath-holding

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ABSTRACT

Until now oxygen was thought to be the leading factor of hypoxic conditions. Whereas now it appears that insulin is the key regulator of hypoxic conditions. Insulin seems to regulate the redox state of the organism and to determine the breakpoint of human breath-holding. This new hypoxia–insulin hypotheses might have major clinical relevance. Besides the clinical relevance, this hypothesis could explain, for the first time, why the training of the diaphragm, among other factors, results in an increase in breath-holding performance.

Elite freedivers/apnea divers are able to reach static breath-holding times to over 6 min. Untrained persons exhibit an unpleasant feeling after more or less a minute. Breath-holding is stopped at the breakpoint. The partial oxygen pressure as well as the carbon dioxide pressure failed to directly influence the breakpoint in earlier studies. The factors that contribute to the breakpoint are still under debate.

Under hypoxic conditions the organism needs more glucose, because it changes from the oxygen consuming pentose phosphate (36ATP/glucose molecule) to the anaerobic glycolytic pathway (2ATP/glucose molecule). Hence insulin, as it promotes the absorption of glucose, is set in the center of interest regarding hypoxic conditions. This paper provides an insulin based model that could explain the changes and interactions in human breath-holding. The correlation between hypoxia and reactive oxygen species (ROS) and their influence on the sympathetic nerve system and hypoxia-inducible factor 1 alpha (HIF-1α) is dealt with. It reviews as well the direct interrelation of HIF-1α and insulin. The depression of insulin secretion through the vagus nerve activation via inspiration is discussed. Furthermore the paper describes the action of insulin on the carotid bodies and the diaphragm and therefore a possible role in respiration pattern.

Freedivers that go over the breakpoint of breath-holding could exhibit seizures and thus the effect of insulin, blood glucose levels and corticosteroids in hippocampal seizures is highlighted.

Introduction

Human breath-holding can be divided into a normoxia part followed by a hypoxia one. In the hypoxia part the desire to breathe becomes stronger. Diaphragmatic movements contribute to this increased desire to take a breath. Over time the diaphragmatic movements normally increase to strong contractions [1].

The factors that determine the breakpoint, where the person stops the voluntary breath-holding, are still under debate. It should be noted that some individuals assert to being able to ignore the breakpoint, which then leads to unconsciousness [2].

Freedivers use water free breath-holding or so called dry static as a training method. Elite freedivers are able to reach times to over 6 min in dry static and that more or less without the mammalian diving reflex. The mammalian diving reflex is strengthened by cold water contacting the face and helps to extend breath-holding as bradycardia, peripheral vasoconstriction and blood shift are evoked.

What can contribute to such times in dry static? Of course an increase in lung inflation results in an increased breath-holding time. Also a reduction in the metabolic rate helps [2].

It was shown that the lung gradually contracts by 200–500 ml/min during breath-holding as a result of the failure to substitute completely the pull out O_2 by CO_2 [3]. As the CO_2 cannot be removed from the alveoli, the partial pressure gradient grows and does not allow the removal of CO_2 from the blood [2]. The consequence is hypercapnia with an acidosis. The shrinkage of the lung volume was ruled out as having an important effect on the breakpoint [4]. The next question is: does partial pressure of O_2 and/or CO_2 have an influence on the breakpoint? Loss of consciousness was estimated in normal adults at arterial partial pressure levels

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of O_2 ~27 mmHg and CO_2 ~90–120 mmHg [5]. Breakpoints in these pressure ranges are described [6]. In order to find out if a special ratio or threshold of the arterial pressure determines the breakpoint, the carotid chemoreceptors were denervated. The denervation of the carotid chemoreceptors fails to prolong breath-holding until unconsciousness [2,7]. But, interestingly, the duration of breath-holding was prolonged [8]. It has been suggested that the chemoreceptors of the diaphragm muscle may contribute to the breakpoint [9]. Breathing an asphyxiating gas mixture directly after breath-holding does not affect the subjects' ability to perform a second breath-hold [10]. This is another fact that underlies the thesis that the breakpoint of breath-holding is not just dependent on blood gas parameters. Parkes concludes that the relaxing of the tonic diaphragm activity makes a second breath-holding after breathing asphyxiating gas mixture possible [2].

The indirect measurement of diaphragmatic electromyogram (EMG) by swallowed catheters showed rhythmic activity near the end of breath-hold [11]. It should be mentioned that it is possible that some tonic and/or almost isometric contraction (almost, because of the shrinkage of lung volume while breath-holding) of the diaphragm are not recorded with this method [2]. The recorded frequency range was within the respiration frequency [2,12]. Together with the persistent respiratory sinus arrhythmia this could demonstrate the presence of some central respiratory rhythm/oscillations during breath-holding [2]. An implication of these findings is that human breath-holding is a voluntary act by holding the chest and suppressing the central respiratory pattern [2]. When talking about the breakpoint, the diaphragm as the main breathing muscle becomes an interesting subject. In an experiment where two atropinized persons were paralyzed using d-tubocurarine and mechanically ventilated, they reported feeling no discomfort or any urge to breath [13]. It should be mentioned that the experiment was interrupted after 4 min and the persons were able to communicate as one arm was protected from d-tubocurarine paralysis via an arterial occlusion [13]. These results could not be reproduced. In a later similar experiment (except the trachea was intubated transnasally), the participants experienced severe dyspnea at the breakpoint and no increase in breath-holding time [14]. However another experiment showed the influence of the diaphragm on the duration of breath-holding.

The bilateral injection of local anesthetic into the phrenic nerve resulted in nearly a doubling of the breath-holding time [15]. The phrenic nerve in cats, dogs and rats carries approximately 30% afferent fibers [16]. A part of these afferents are chemoreceptors [16]. Also the vagal nerve contains afferents from the diaphragm. A blockage of the vagus, the glossopharyngeal nerve, the afferents from the carotid, aortic chemoreceptors and from the lung eases the distress of breath-holding and trebled the extent of breath-holding time [2,17]. Parkes postulates that the contraction of the diaphragm leads to muscle fatigue, which then could stimulate the chemoreceptors and thus contribute to the breakpoint [2]. The relaxation of the diaphragm minimizes the stimulation of the proprioceptors and the chemoreceptors through the increased blood flow [2]. Parkes concludes that there could still be a missing element as neither phrenic nor vagal blockage allows breath-holding to unconsciousness [2].

It should be mentioned that in freediving competitions breath-holding till loss of consciousness is seen. This also applies to dry static training. Breath-holding is a voluntary act and the urge to breathe is accomplished by a strong emotional feeling. It seems to be possible to consciously go over the breakpoint. Higher brain centers such as the amygdala and the connected brain structures are therefore highly interesting. This is because it is well accepted that mental training, as well as yoga, can influence the amygdala and therefore the mental state. Most of the competitive freedivers invest a lot of time in training their mental states to achieve longer times thus inferring a connection between higher brain centers and autonomic nervous system in breath-holding.

The brain areas that are involved in the loss of consciousness and the neurological disorders could give hints to which factors contribute to the breakpoint. These can include loss of motor control (LMC) and/or black out (BO) that can occur during freediving.

The hippocampus is well known for its susceptibility to hypoxia and for its major role in hypoxia-induced seizures. Thus it becomes a special region of interest.

Besides the pancreas, the adult granule neurons of the hippocampus are able to produce insulin [18]. The hippocampus is a highly active metabolic tissue. Particularly in a hypoxic state when glycolysis shifts the energy balance from 36 ATP to 2 ATP per glucose molecule and a higher amount of glucose is needed to sustain metabolic balance. Here insulin could play a role, since it induces extra glucose uptake via the insulin-dependent glucose transporter GLUT4, in addition to the insulin-independent glucose transporters GLUT1 and GLUT3 [19,20]. It is accepted that under hypoxic conditions the brain switches from the oxygen consuming pentose phosphate to the glycolytic pathway [21]. Furthermore it has been shown that depending on the paradigm used and brain regions during activation under normoxia, the nonoxidative metabolism increases more than the oxidative one [22]. This strengthens the role of glucose as a major fuel for the brain. Insulin has been shown to reduce hippocampal injury after ischemia [23]. Therefore insulin is a potential key regulator in hypoxic conditions when considering these facts.

Hypotheses

The first subsection of this part is dealing with hypoxic conditions and their influence on insulin levels. The correlation between hypoxia, ROS and HIF-1α and then the correlation between HIF-1α and insulin is discussed. Giving evidence that the insulin level should increase with the rise of HIF-1α in hypoxic conditions. Subsequently followed by discussing the influence of insulin on the carotid bodies and the diaphragm in the second subsection. This subsection provides an explanation for the diaphragmatic movements near the end of breath-holding performance. That means synonymously the setting of the breakpoint in order to start breathing and escape the hypoxic condition. In the second subsection also the interrelation between diaphragm contraction, triggered pulmonary stretch receptors, activated afferent vagus nerve and diminished insulin secretion is considered. An explanation for increased breath-holding performance through diaphragm training is given. The third subsection explains the neurological disorders, which could be observed, when freedivers ignore the breakpoint. Especially the effect of insulin on the intracellular calcium levels via modification of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) is described.

Correlation between hypoxia and insulin through ROS and HIF-1α

Breath-holding induces hypoxic stress. In this phase ROS level increases [24,25] (Fig. 1; Arrow 1a). Cells protect themselves against ROS via enzymes such as glutathione peroxidase, superoxide dismutase, catalase, lactoperoxidase, etc. High amounts of ROS can damage cell structures and can lead to apoptosis (Fig. 1; Arrow 12). Factors that can conduce ROS production are muscle contractions (Fig. 1; Arrow 1b), decreased O_2 tension, increased CO_2 tension, decreased cellular pH and also increased muscle temperature [26,27]. The source of ROS is still not totally clear. Possible sources for the production of ROS in the skeletal muscle are processes of the mitochondrial electron transport chain,

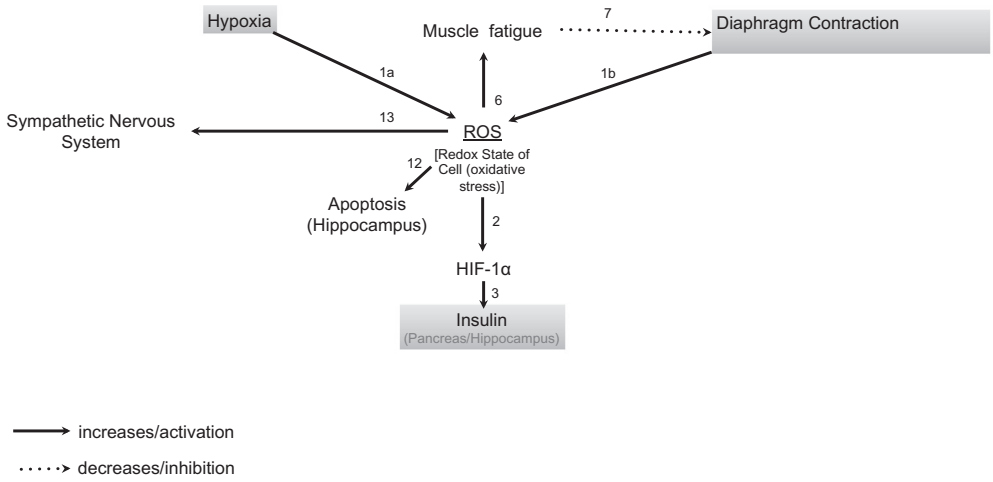

Fig. 1. Correlation between hypoxia and insulin through ROS and HIF-1α.

nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, phospholipase A_2 (PLA_2) and xanthine oxidase [25]. The effect of ROS on the musculature is a dose dependent influence on the muscle force. In low concentration it increases the muscle force, at high levels it decreases it [28,29] (Fig. 1; Arrow 6, 7). Therefore the redox state of the muscle could be a regulation tool of the isometric muscle force [30]. A possible explanation is that increased ROS production can alter the calcium release from the sarcoplasmic reticulum and also the calcium sensitivity of myofilaments [28,30]. It has been shown that antioxidants can prevent muscular fatigue [31]. The ROS-scavenger N-acetylcysteine [32], as a supporter for glutathione resynthesis, postpones the muscle fatigue of an in situ prepared diaphragm [31]. These findings are in line with the reports of elite freedivers that antioxidants like vitamin E, C, resveratrol, methylsulfonylmethane, NAC, coenzyme Q_{10} etc. have a positive effect on their (static) performance. It should be mentioned that ROS also activates the sympathetic nervous system [33,34] (Fig. 1; Arrow 13). Moreover, increased ROS production in the brainstem supports the neural mechanism of hypertension [33].

Heart rate variability (HRV) and skin conductance response (SCR) measurements in elite freedivers during dry static displayed that autonomic control on heart rate and skin conductance are divergently controlled during breath-holding as heart rate did not exhibit changes in sympathetic tone [1]. This could be due to a pronounced effect of emotional control created by mental training [1].

If the increase of ROS is claimed to be part of the body's reaction to hypoxia, there should be a correlation to HIF1-α, the key regulator in the response of cellular and systemic hypoxia. Under normoxia, HIF1-α is hydroxylated [35,36]. Under hypoxic conditions, it is stabilized, forms together with aryl hydrocarbon receptor nuclear translocator (ARNT), the DNA-binding transcription factor HIF [24,36]. Brunelle et al. showed in a cell model that hydrogen peroxide (H_2O_2) of the ROS population is required for the stabilization of HIF-1α since an increase in glutathione peroxidase decreases the stabilization of HIF-1α [24]. They also discovered that the stabilization of HIF-1α is dependent on the mitochondrial electron transport chain, but independent of intracellular oxygen levels and oxidative phosphorylation [24]. Leading to the conclusion that the redox state of the cell is the crucial factor for the stabilization of HIF1-α [24] (Fig. 1; Arrow 2). The stabilized HIF1-α forms together with ARNT and co-factors a transcriptional complex that activates genes like SLC2A1 (glycolysis), VEGFA (angiogenesis) and EPO (erythropoiesis) and so adapting the cell to hypoxic conditions [36]. This would partly contradict the observation that antioxidant elements like NAC improve static performances on a long term basis. They reduce the stabilization of HIF1-α and the adaption of the body to hypoxic condition depends on HIF1-α. Recently it has been demonstrated that NAC not only reduces ROS, but may also stabilize HIF1-α by raising its interaction with the chaperon protein Hsp90 [37].

Increased level of hemoglobin should improve breath-holding performances in top athletes as more oxygen can be stored. But there are no consistent data about the hemoglobin level in breath-hold divers [38]. One study showed an increase of 24% in erythropoietin levels [39], while another did not show any difference between breath-holding divers and normal values [32]. Further investigations with top athletes are needed.

If the previously mentioned hypothesis that insulin could play a regulation role in hypoxic stress conditions should be verified, then there has to be a correlation of insulin and HIF1-α. Cheng et al. proved the interrelation of HIF1-α and the pancreatic β-cells function [40]. Glucose-stimulated ATP generation and islet function was impaired by decreased HIF1-α level in Type 2 diabetes (T2D) [40]. Increased HIF1-α level increased ARNT expression and also improved insulin secretion [40] (Fig. 1; Arrow 3). Another recently done study pointed out the connection of the severity of obstructive sleep apnea syndrome (OSAS), glucose and blood pressure variability in non-diabetic subjects [41]. Furthermore the minimum saturation of hemoglobin with oxygen (SpO_2) was correlated with glucose levels [41]. Although the mechanism of this phenomenon is still not clear, it could be a hint for the hypothesis of the relationship between hypoxia and insulin. It should be mentioned that in the case of OSAS more variables like the dysregulation of the hypothalamic-pituitary (HPA) axis could contribute to the impaired glucose metabolism.

Insulin effects on respiration through influence on diaphragm and carotid bodies – decreased insulin secretion via inspiration activated afferent vagus nerve

During breath-holding the diaphragm is held more or less isometrically contracted at the end of the inspiration phase. During inspiration the afferent parts of the vagal nerve is activated via the pulmonary stretch receptors (Fig. 2; Arrow 4a). These afferent fibers rise to the medullary inspiratory center and pontine respiratory group (PRG) of the rostral dorsal lateral pons. As a reaction, the inspiratory area is inhibited and allows expiration to take place,

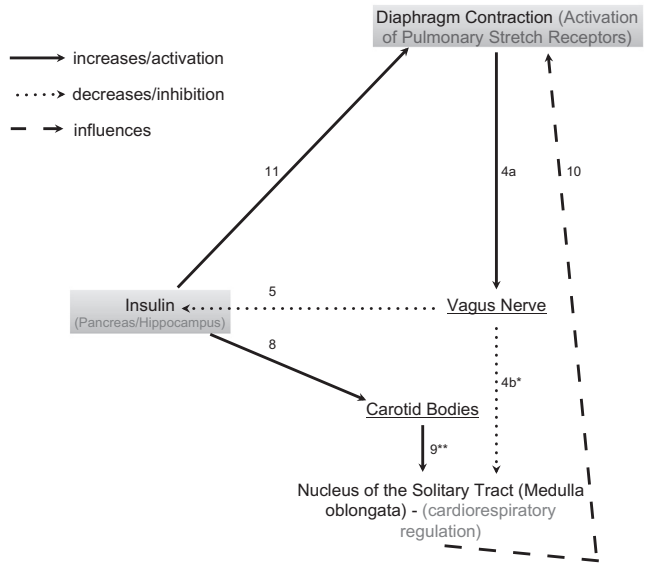

Fig. 2. Insulin effects on respiration through influence on diaphragm and carotid bodies – decreased insulin secretion via inspiration activated afferent vagus nerve.

known as Hering–Breuer reflex. It should be mentioned that vagal and pontine stimulation seems to be mediated by the same medullary circuits, which control the onset and termination of inspiration [42,43].

In OSAS patients, treated with hyperbaric therapy, which lead to elevated pressure in the lung, the Hering–Breuer reflex occurs and results in apneas. As demonstrated previously, strong continuous vagal stimulation arrests the breathing rhythm in the post-inspiration phase [44], thus protecting the lung from over stretching.

The vagal afferents of the lung also have an inhibitory influence on the cardiac vagal motor neurons (CVM) in the nucleus ambiguus (NA) and the dorsal vagal nucleus (DMVN). This results in tachycardia, known as respiratory sinus arrhythmia (RSA). During breath-holding the RSA does persist [2,45], indicating the activity of the afferent vagus nerve.

Curry could show that afferent vagal stimulation in in situ brain–pancreas preparations of cervical vagotomized rats results in inhibition of insulin secretion accompanied by apnea [46]. This concludes that afferent vagal stimulation through inspiration can result in a decreased insulin secretion (Fig. 2; Arrow 5). That makes sense relating to the hypoxia–insulin hypothesis.

During inspiration there should normally be no lack of oxygen and therefore no need for extra insulin. But if there were a lack of oxygen, the organism needs an escape mechanism. This could be accomplished by the previously described rise of ROS, and therefore, via HIF1-α, an increase in the insulin level. To fulfill the thesis, an insulin sensitive receptor related to the respiratory system must then be postulated. Recently it has been shown that insulin triggers the carotid bodies in vagotomized and euglycemic clamped rats [47] (Fig. 2; Arrow 8). Intracarotid bolus of insulin increases dose dependent the ventilation via the carotid bodies [47] (Fig. 2; Arrow 10). This is in line with the aforementioned prolonged breath-holding time by denervation of carotid bodies (see Introduction). So insulin could contribute to the breakpoint of breath-holding and increase the urge to breath. The carotid bodies are known to respond to hypoxia, causing hyperventilation and activation of the sympathetic system [47,48]. Bilateral cutting of the carotid sinus nerves abolished the increase of ventilation evoked by hypoxia (assessed as common carotid artery occlusion) [47]. It should be noted that the model of ischemia to produce a hypoxic state could imply more variables than just hypoxic conditions.

The afferents of the carotid body and also the pulmonary stretch receptors enter the ponto-medullary network. This network is essential for respiratory rhythm and motor pattern generation, via the nucleus of the solitary tract (NTS) and the ventrolateral medulla [49,50] (Fig. 2; Arrow 4b*, 9**). The NTS is the entry station of afferents that affect the motor pattern of breathing and the regulation of lung volume by coordination of diaphragmatic, intercostal, abdominal, laryngeal and other muscular outputs [43,49] (Fig. 2; Arrow10). Hence making insulin as a respiratory pattern regulator and thus as a hypoxic stress regulator highly interesting. The previously mentioned findings could explain the diaphragmatic movements at the end of the hypoxic phase of breath-holding and also the muscular contractions of other respiratory muscles.

There seems to be another possible escape mechanism from breath-holding. Recently a neuro-vascular proximity in the diaphragm muscle of adult mice was shown to be apparent for the whole muscle [51]. Correa and Segal hypothesize that the correlation between motor nerves and arteriolar supply may promote local perfusion of the diaphragm muscle according to the recruitment of its muscle fibers [51]. Espinosa et al. showed that insulin produces a fast (<1 s) and transient Ca^{2+} influx in rodent muscles, which could be nearly completely blocked by a Ca^{2+} free medium [52]. This should be given special consideration due to the fact that calcium levels regulate contractile muscle function. Therefore their results should be taken into consideration, even though they used higher insulin plasma levels than normally found in rodents. Brotto et al. demonstrated that decreased diaphragm function in diabetic rats (streptozotocin induced) was completely reversible by insulin [53]. Putting these findings together, insulin could have an additional effect on action potentials of motor neurons to the diaphragm (Fig. 2; Arrow 11). Insulin could also influence the respiratory center in this way. It should be noted that the results from Brotto et al. were from day 4, week 4, week 8 and week 14 after the diabetic onset. They also showed pathological changes in the muscle fibers (additional gamma-tropomyosin) [53]. Although these findings are interesting, this research does not explicitly show a direct stimulatory effect of insulin. Further research is implicitly needed. Particularly it could confirm the postulation from Parkes that the diaphragm and its chemoreceptors could contribute to the breakpoint of breath-holding [2].

Considering that some freedivers are able to fight or ignore the diaphragmatic movements and can go voluntarily over the breakpoint, this could be a hint for an explicit muscle action induced or accomplished by activation of local chemoreceptors. It should not be forgotten that the insulin action on the carotid bodies is dose dependent and also could explain this observation. Ribeiro et al. did not find a dose dependent relationship between insulin receptor phosphorylation and high levels of insulin [47]. They mentioned that at high insulin levels the receptors may be saturated and induce a desensitization by either decreasing tyrosine kinase activity or by promoting insulin receptor endocytosis and degradation [47].

Insulin influences on hippocampal AMPAR modification and the effects on intracellular Ca^{2+} levels and neurological disorders – additional roles of corticosteroids and blood glucose levels

As previously described, the vagal afferents are triggered through activation of the stretch receptors of the lung, which leads to a decreased insulin secretion. Mechanical ventilation, especially high pressure ventilation, was shown to trigger hippocampal apoptosis in mice by vagal and dopaminergic pathways [54]. The study was published in 2013 and the authors stated that the pathologic

mechanism has not yet been discovered. Taking the hypoxia–insulin hypothesis under consideration, there could be a disturbance in the insulin level. Anarkooli et al. demonstrated that insulin has an antiapoptotic effect on the hippocampus of streptozotocin induced diabetic rats [55] (Fig. 3; Arrow 15a). Streptozotocin has a toxic effect on pancreatic beta-cells and therefore could induce lower levels of insulin (Type 1 diabetes) that could lead to damage of the hippocampal cells. It is generally suggested that diabetic patients have a higher incidence of cognitive deficits and memory impairment [56,57]. Neurocognitive disturbance in critically ill and mechanically ventilated patients is also well known [54]. It was shown that insulin induces long-term depression (LTD) on the hippocampus [58]. This could be another explanation for the observed cognitive deficits besides the apoptotic effects in the hippocampus. Long-term depression is thought to be a part of spatial memory formation processes. Huang et al. exhibit that "rapid endocytotic removal of postsynaptic glutamate receptor 2 subunits (GluR2) is involved in the process of insulin-LTD formation" [58].

The AMPA receptor with the subunit GluR2 is, in general, impermeable for Ca^{2+}. It is known that Ca^{2+} fluxes are involved in the genesis of epileptic seizures (Fig. 3; Arrow 17). Known as paroxysmal depolarization (PDS) shift, it starts with a calcium mediated depolarization of the neuron that causes an opening of the voltage gated sodium channels, which then leads to action potentials. As a consequence the calcium dependent potassium channels open and/or GABA activated chloride influx occurs, resulting in a hyperpolarization. But increased intracellular calcium could sustain the paroxysmal depolarization shift [59]. It is assumed that PDS is mainly produced by AMPARs.

Sanchez et al. found out that the susceptibleness for hypoxia induced seizures in the neonatal rat hippocampus is highest when the overall amount of AMPA receptors is high and the expression of GluR2 is relatively low [60]. As already discussed, insulin induces an endocytotic removal of GluR2 (Fig. 3; Arrow 15b). Via insulin induced hypoglycemia there is also an increase of glutamate receptors in the cerebral cortex in rats [61]. This study did not look up changes in the hippocampus and thus further investigations for that specific region are needed. Not only hypoglycemia, also hyperglycemia can harm the hippocampal cells via changes in calcium levels. Rytter et al. showed that hyperglycemia could elevate ROS in murine hippocampal slice cultures resulting in an intracellular calcium level increase [62] (Fig. 3; Arrow 21, 16). Hyperglycemia causes a lowered threshold for seizures [63]. Taking these findings together, insulin, perhaps only in combination with an altered blood glucose level, could provoke seizures in certain conditions according to the findings of Sanchez et al. Another study that underlies the importance of glutamate receptors, especially of the AMPA receptors, was done by Ceolin et al. They found out that the anti-epileptic drug, perampanel, a non-competitive AMPA receptor antagonist, reduces AMPA induced calcium signals in the hippocampus [64].

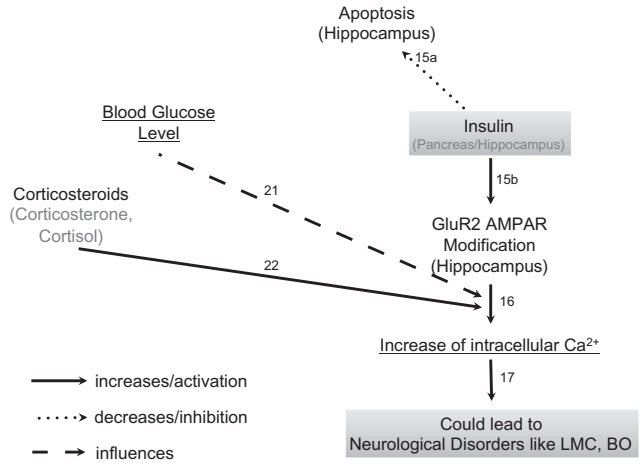

Fig. 3. Insulin influences on hippocampal AMPAR modification and the effects on intracellular Ca^{2+} levels and neurological disorders – additional roles of corticosteroids and blood glucose levels.

When speaking about seizure susceptibility in combination with breath-holding, the possible impact of the sympathetic nervous system could be important. It was antecedently demonstrated that preventing an increase in the level of corticosteroids during ischemia decreases the possibility of seizure occurrence [65] (Fig. 3; Arrow 22).

Kruger et al. did show that the pretreatment with the corticosterone synthesis inhibitor metyrapone decreases the incidence of seizures after ischemia in rats [65]. Furthermore they demonstrated that pretreatment with metyrapone protects against loss in synaptic function in the hippocampus [65]. Both effects occur in the absence of a change in the blood glucose levels [65]. Sebastian et al. demonstrated that acute physical stress, and therefore increased corticosterone, decreases GluR2 in the CA3 region of the rat's hippocampus [66] (Fig. 3; Arrow 22, 16). This region is thought to be the pacemaker of the hippocampus and is associated with epileptiform activity. The region CA3 is also thought to be important for the retrieval of short-term spatial and/or novel information [67]. Hence the relationship between insulin, corticosteroids and blood glucose level is highly interesting regarding the neurological disorders observed with breath-holding.

The previous subsection indicates the clinical relevance of the hypoxia–insulin hypothesis and the need for further research.

Vagal nerve stimulation (VNS) is a unique epilepsy treatment and therefore a further hint for the hypoxia–insulin hypothesis. Also current studies state that the mechanism of this therapy is not fully elucidated [68] and the hypoxia–insulin hypothesis could be an explanation. As previously described the afferents of the vagus nerve travel to the NTS. The NTS has connections to locus coeruleus (LC) in the pons, raphe nuclei in the brainstem and other regions of the central nervous system like medulla, forebrain, limbic system (central amygdaloid nucleus), etc. [68]. Acute (<3 days) VNS increases LC activity [69] and increases downstream release of norepinephrine into the amygdala, hippocampus and prefrontal cortex [70,71]. With chronic (>14 days) VNS, the baseline activity of the LC and also of the dorsal raphe nucleus (DRN) is nearly doubled [69]. For the increased activity in the DRN the LC has to be intact [68,72]. The highest amounts of serotonergic neurons of the brain are found in the DRN [68]. Areas that are influenced by these neurons are the striatum, the neocortex, the substantia nigra and the cerebellum [68]. It has been shown that an increase in norepinephrine or serotonin levels has anticonvulsant effects [68,73]. This has been suggested to be the explanation for the anti-epileptic effects of VNS. Here the LC seems to be the critical region in the seizure suppressing effect of VNS [68]. The correlation between the afferent vagus activation and insulin suppression was not mentioned. In the late 1970s, it was shown in an isolated perfused rat islets model that norepinephrine depresses insulin secretion [74]. This could also be expected for the insulin production in the hippocampus, as insulin producing cells are considered to have a high rate of "functional analogues and evolved from a common ancestral insulin-producing neuron" [18,75]. Here the circle for the hypoxia–insulin hypothesis closes.

Conclusion

The insulin based model (Fig. 4) could explain the changes and interactions in human breath-holding. In hypoxic conditions, it appears as insulin secretion rises with increased HIF-α levels.

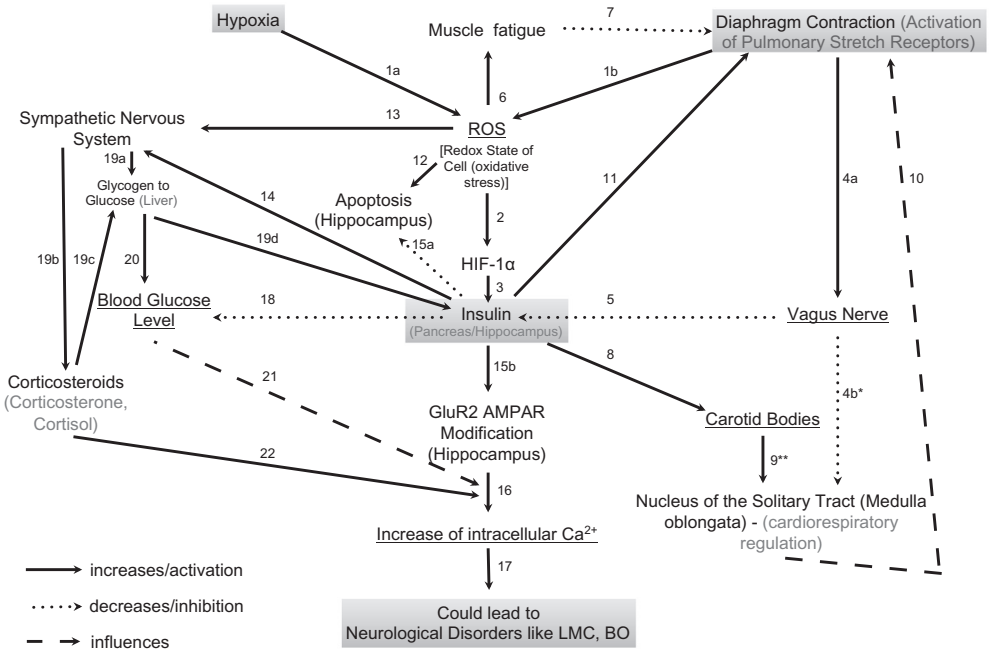

Fig. 4. "Hypoxia–Insulin-Hypotheses". While breath-holding, the body is under a hypoxic condition and builds up ROS [Arrow 1a, 1b]. As a consequence the sympathetic nerve system is activated and also the accumulation of HIF-1α is triggered [Arrow 13]. HIF-1α enhances the secretion of insulin [Arrow 3]. The increase of insulin triggers the carotid bodies and could lead to a higher contractibility of the diaphragm [Arrow 8, 9**, 10, 11]. During breath-holding, the diaphragm is contracted and therefore the pulmonary stretch receptors are activated. Hence the afferent properties of the vagal nerve are stimulated and a decrease in insulin secretion is evoked [Arrow 4a, 5]. Just as through the vagal nerve activation, an induced apnea appears. The subsequent fatigue of the diaphragm leads to a loss of contraction force of the muscle [Arrow 6]. As a result the afferent vagal activation ceases and the insulin amount furthermore increases, so that a contraction can easily follow the relaxation. This could lead to the so called "contractions of the diaphragm" in freediving. In the brain, insulin rapidly induces an endocytotic postsynaptic removal of the glutamate receptor 2 subunit (GluR2) of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) in the hippocampus [Arrow 15b]. This event, in combination with the activation of the sympathetic system and therefore changes in the levels of corticosteroids like corticosterone, cortisol, and also possible changes in blood glucose level, could lead to an increased calcium ion influx [Arrow 14, 16, 19a, 19b, 19b, 19c, 19d, 20, 21, 22]. Thus providing an explanation for the observed neurological problems like dizziness, tremors, coordination problems, seizure and loss of consciousness, also known as loss of motor control (LMC) and/or black out (BO)" that can occur during freediving [Arrow 17]. It is well accepted that the susceptibility for these neurological problems is also affected by insulin induced hypoglycemia, just as with hyperglycemia.

Insulin triggers the carotid bodies, which should result in diaphragmatic movements. Also a direct influence of insulin, and also ROS, on the muscle action of the diaphragm via a neuro-vascular proximity seems possible. Both mechanisms could cause the breakpoint of breath-holding, helping the body to escape the hypoxic condition via breathing. During breath-holding, insulin secretion is decreased through the activated afferent vagus nerve via the pulmonary stretch receptors. As long as the diaphragm can be hold contracted the stretch receptors are triggered and therefore the insulin secretion is diminished. Hence explaining why training of breath-holding, or better the training of the diaphragm, results in increased breath-holding performances. As soon as the muscle force vanishes insulin level rises and the breakpoint is set. The observed LMC and/or BO of freedivers that go over the breakpoint (ignoring the diaphragmatic movements) could be explained by calcium influx in the hippocampus. This influx could be caused by AMPA receptor modification through insulin.

So it seems that insulin is more or less a key regulator in hypoxic conditions. It has to be taken into consideration that under hypoxic situations the organism has to switch to anaerobic metabolism and therefore is in need of more glucose. Hence insulin is put into the spotlight of interest when it comes to metabolism under hypoxic conditions and a relative maintenance of a survival fitting redox state. As generally accepted the redox state of the cell is crucial for the survival, cell processes are electro-chemically mediated. Thus, setting the redox state of the cell, meaning the oxidative stress level, into the sensor role in hypoxic situations. Further investigations are needed as the presumed correlations in sum are not yet shown and different experimental settings make their comparison difficult and somewhat questionable.

Conflict of interest

None.

References

[1] Laurino M, Menicucci D, Mastorci F, et al. Mind-body relationships in elite apnea divers during breath holding: a study of autonomic responses to acute hypoxemia. Front Neuroeng 2012;5:4.
[2] Parkes MJ. Breath-holding and its breakpoint. Exp Physiol 2006;91:1–15.
[3] Hong SK, Lin YC, Lally DA, et al. Alveolar gas exchanges and cardiovascular functions during breath holding with air. J Appl Physiol 1971;30:540–7.
[4] Godfrey S, Edwards RH, Warrell DA. The influence of lung shrinkage on breath holding time. Q J Exp Physiol Cogn Med Sci 1969;54:129–40.
[5] Nunn JF. Mechanisms of pulmonary ventilation. In: Applied respiratory physiology. London: Butterworths; 1987.
[6] Klocke FJ, Rahn H. Breath holding after breathing of oxygen. J Appl Physiol 1959;14:689–93.
[7] Davidson JT, Whipp BJ, Wasserman K, Koyal SN, Lugliani R. Role of the carotid bodies in breath-holding. N Engl J Med 1974;290:819–22.
[8] Honda Y, Hashizume I, Kimura H, Severinghuas JW. Bilateral carotid body resection in man enhances hypoxic tachycardia. Jpn J Physiol 1988;38:917–28.
[9] Road JD. Phrenic afferents and ventilatory control. Lung 1990;168:137–49.
[10] Flume PA, Eldridge FL, Edwards LJ, Houser LM. The fowler breathholding study revisited – continuous rating of respiratory sensation. Respir Physiol 1994;95:53–66.
[11] Whitelaw WA, Mcbride B, Ford GT. Effect of lung-volume on breath holding. J Appl Physiol 1987;62:1962–9.
[12] Agostoni E. Diaphragm activity during breath holding – factors related to its onset. J Appl Phys 1963;18:30–6.
[13] Campbell EJ, Freedman S, Clark TJH, Robson JG, Norman J. Effect of muscular paralysis induced by tubocurarine on duration and sensation of breath-holding. Clin Sci 1967;32:425.
[14] Gandevia SC, Killian K, Mckenzie DK, et al. Respiratory sensations, cardiovascular control, kinaesthesia and transcranial stimulation during paralysis in humans. J Physiol Lond 1993;470:85–107.

[15] Noble MIM, Eisele JH, Frankel HL, Else W, Guz A. Role of diaphragm in sensation of holding breath. Clin. Sci. 1971;41:275.
[16] Langford LA, Schmidt RF. An electron microscopic analysis of the left phrenic nerve in the rat. Anat Rec 1983;205:207–13.
[17] Guz A, Noble MIM, Trenchar D, Eisele J. Effect of selective nerve blocks or neurological disease on respiratory sensations. Eur J Clin Invest 1970;1:134.
[18] Kuwabara T, Kagalwala MN, Onuma Y, et al. Insulin biosynthesis in neuronal progenitors derived from adult hippocampus and the olfactory bulb. EMBO Mol Med 2011;3:742–54.
[19] Grillo CA, Piroli GG, Hendry RM, Reagan LP. Insulin-stimulated translocation of GLUT4 to the plasma membrane in rat hippocampus is PI3-kinase dependent. Brain Res 2009;1296:35–45.
[20] Emmanuel Y, Cochlin LE, Tyler DJ, de Jager CA, David Smith A, Clarke K. Human hippocampal energy metabolism is impaired during cognitive activity in a lipid infusion model of insulin resistance. Brain Behav 2013;3:134–44.
[21] Dienel GA. Brain lactate metabolism: the discoveries and the controversies. J Cereb Blood Flow Metab 2012;32:1107–38.
[22] Dienel GA, Cruz NF. Imaging brain activation simple pictures of complex biology. Ann N Y Acad Sci 2008;1147:139–70.
[23] Collino M, Aragno M, Castiglia S, et al. Insulin reduces cerebral ischemia/reperfusion injury in the hippocampus of diabetic rats: a role for glycogen synthase kinase-3beta. Diabetes 2009;58:235–42.
[24] Brunelle JK, Bell EL, Quesada NM, et al. Oxygen sensing requires mitochondrial ROS but not oxidative phosphorylation. Cell Metab 2005;1:409–14.
[25] Powers SK, Nelson WB, Hudson MB. Exercise-induced oxidative stress in humans: cause and consequences. Free Radic Biol Med 2011;51:942–50.
[26] Arbogast S, Reid MB. Oxidant activity in skeletal muscle fibers is influenced by temperature, CO(2) level, and muscle-derived nitric oxide. Am J Physiol-Reg I 2004:R698–705.
[27] Clanton TL. Hypoxia-induced reactive oxygen species formation in skeletal muscle. J Appl Physiol 2007;102:2379–88.
[28] Andrade FH, Reid MB, Westerblad H. Contractile response of skeletal muscle to low peroxide concentrations: myofibrillar calcium sensitivity as a likely target for redox-modulation. FASEB J 2001;15:309–11.
[29] Haycock JW, Jones P, Harris JB, Mantle D. Differential susceptibility of human skeletal muscle proteins to free radical induced oxidative damage: a histochemical, immunocytochemical and electron microscopical study in vitro. Acta Neuropathol 1996;92:331–40.
[30] Reid MB, Khawli FA, Moody MR. Reactive oxygen in skeletal-muscle. 3. Contractility of unfatigued muscle. J Appl Physiol 1993;75:1081–7.
[31] Reid MB, Stokic DS, Koch SM, Khawli FA, Leis AA. N-acetylcysteine inhibits muscle fatigue in humans. J Clin Invest 1994;94:2468–74.
[32] Prommer N, Ehrmann U, Schmidt W, Steinacker JM, Radermacher P, Muth CM. Total haemoglobin mass and spleen contraction: a study on competitive apnea divers, non-diving athletes and untrained control subjects. Eur J Appl Physiol 2007;101:753–9.
[33] Hirooka Y, Kishi T, Sakai K, Takeshita A, Sunagawa K. Imbalance of central nitric oxide and reactive oxygen species in the regulation of sympathetic activity and neural mechanisms of hypertension. Am J Physiol Regul Integr Comp Physiol 2011;300:R818–26.
[34] Kishi T, Hirooka Y, Kimura Y, Ito K, Shimokawa H, Takeshita A. Increased reactive oxygen species in rostral ventrolateral medulla contribute to neural mechanisms of hypertension in stroke-prone spontaneously hypertensive rats. Circulation 2004;109:2357–62.
[35] Ivan M, Kondo K, Yang H, et al. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. Science 2001;292:464–8.
[36] Greer SN, Metcalf JL, Wang Y, Ohh M. The updated biology of hypoxia-inducible factor. EMBO J 2012;31:2448–60.
[37] Zhang Z, Yan J, Taheri S, Liu KJ, Shi H. Hypoxia-inducible factor 1 contributes to N-acetylcysteine's protection in stroke. Free Radic Biol Med 2013;68C:8–21.
[38] Lindholm P, Lundgren CE. The physiology and pathophysiology of human breath-hold diving. J Appl Physiol (1985) 2009;106:284–92.
[39] de Bruijn R, Richardson M, Schagatay E. Increased erythropoietin concentration after repeated apneas in humans. Eur J Appl Physiol 2008;102:609–13.
[40] Cheng K, Ho K, Stokes R, et al. Hypoxia-inducible factor-1alpha regulates beta cell function in mouse and human islets. J Clin Invest 2010;120:2171–83.
[41] Kallianos A, Trakada G, Papaioannou T, et al. Glucose and arterial blood pressure variability in obstructive sleep apnea syndrome. Eur Rev Med Pharmacol Sci 2013;17:1932–7.
[42] Okazaki M, Takeda R, Yamazaki H, Haji A. Synaptic mechanisms of inspiratory off-switching evoked by pontine pneumotaxic stimulation in cats. Neurosci Res 2002;44:101–10.
[43] Lindsey BG, Rybak IA, Smith JC. Computational models and emergent properties of respiratory neural networks. Compr Physiol 2012;2:1619–70.
[44] Hayashi F, Coles SK, McCrimmon DR. Respiratory neurons mediating the Breuer–Hering reflex prolongation of expiration in rat. J Neurosci 1996;16:6526–36.
[45] Cooper HE, Parkes MJ, Clutton-Brock TH. CO_2-dependent components of sinus arrhythmia from the start of breath holding in humans. Am J Physiol Heart Circ Physiol 2003;285:H841–8.
[46] Curry DL. Reflex inhibition of insulin secretion: vagus nerve involvement via CNS. Am J Physiol 1984;247:E827–32.
[47] Ribeiro MJ, Sacramento JF, Gonzalez C, Guarino MP, Monteiro EC, Conde SV. Carotid body denervation prevents the development of insulin resistance and hypertension induced by hypercaloric diets. Diabetes 2013;62:2905–16.
[48] Marshall JM. Peripheral chemoreceptors and cardiovascular regulation. Physiol Rev 1994;74:543–94.
[49] Kubin L, Alheid GF, Zuperku EJ, McCrimmon DR. Central pathways of pulmonary and lower airway vagal afferents. J Appl Physiol (1985) 2006;101:618–27.
[50] Lipski J, McAllen RM, Trzebski A. Carotid baroreceptor and chemoreceptor inputs onto single medullary neurones. Brain Res 1976;107:132–6.
[51] Correa D, Segal SS. Neurovascular proximity in the diaphragm muscle of adult mice. Microcirculation 2012;19:306–15.
[52] Espinosa A, Estrada M, Jaimovich E. IGF-I and insulin induce different intracellular calcium signals in skeletal muscle cells. J Endocrinol 2004;182:339–52.
[53] Brotto M, Brotto L, Jin JP, Nosek TM, Romani A. Temporal adaptive changes in contractility and fatigability of diaphragm muscles from streptozotocin-diabetic rats. J Biomed Biotechnol 2010;2010:931903.
[54] Gonzalez-Lopez A, Lopez-Alonso I, Aguirre A, et al. Mechanical ventilation triggers hippocampal apoptosis by vagal and dopaminergic pathways. Am J Respir Crit Care Med 2013;188:693–702.
[55] Anarkooli IJ, Sankian M, Vahedi F, Bonakdaran S, Varasteh AR, Haghir H. Evaluation of insulin and ascorbic acid effects on expression of Bcl-2 family proteins and caspase-3 activity in hippocampus of STZ-induced diabetic rats. Cell Mol Neurobiol 2009;29:133–40.
[56] Biessels GJ, Kamal A, Urban IJ, Spruijt BM, Erkelens DW, Gispen WH. Water maze learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: effects of insulin treatment. Brain Res 1998;800:125–35.
[57] Biessels GJ, Kappelle AC, Bravenboer B, Erkelens DW, Gispen WH. Cerebral function in diabetes mellitus. Diabetologia 1994;37:643–50.
[58] Huang CC, Lee CC, Hsu KS. An investigation into signal transduction mechanisms involved in insulin-induced long-term depression in the CA1 region of the hippocampus. J Neurochem 2004;89:217–31.
[59] Ure A, Altrup U. Block of spontaneous termination of paroxysmal depolarizations by forskolin (buccal ganglia, Helix pomatia). Neurosci Lett 2006;392:10–5.
[60] Sanchez RM, Koh S, Rio C, et al. Decreased glutamate receptor 2 expression and enhanced epileptogenesis in immature rat hippocampus after perinatal hypoxia-induced seizures. J Neurosci 2001;21:8154–63.
[61] Joseph A, Antony S, Paulose CS. Increased glutamate receptor gene expression in the cerebral cortex of insulin induced hypoglycemic and streptozotocin-induced diabetic rats. Neuroscience 2008;156:298–304.
[62] Rytter A, Cardoso CMP, Johansson P, et al. The temperature dependence and involvement of mitochondria permeability transition and caspase activation in damage to organotypic hippocampal slices following in vitro ischemia. J Neurochem 2005;95:1108–17.
[63] Huang CW, Cheng JT, Tsai JJ, Wu SN, Huang CC. Diabetic hyperglycemia aggravates seizures and status epilepticus-induced hippocampal damage. Neurotox Res 2009;15:71–81.
[64] Ceolin L, Bortolotto ZA, Bannister N, Collingridge GL, Lodge D, Volianskis A. A novel anti-epileptic agent, perampanel, selectively inhibits AMPA receptor-mediated synaptic transmission in the hippocampus. Neurochem Int 2012;61:517–22.
[65] Krugers HJ, Maslam S, Korf J, Joels M, Holsboer F. The corticosterone synthesis inhibitor metyrapone prevents hypoxia/ischemia-induced loss of synaptic function in the rat hippocampus editorial comment. Stroke 2000;31:1162–72.
[66] Sebastian V, Estil JB, Chen D, Schrott LM, Serrano PA. Acute physiological stress promotes clustering of synaptic markers and alters spine morphology in the hippocampus. PLoS One 2013;8:e79077.
[67] Kesner RP. A process analysis of the CA3 subregion of the hippocampus. Front Cell Neurosci 2013;7:78.
[68] Krahl SE, Clark KB. Vagus nerve stimulation for epilepsy: a review of central mechanisms. Surg Neurol Int 2012;3:S255–9.
[69] Dorr AE, Debonnel G. Effect of vagus nerve stimulation on serotonergic and noradrenergic transmission. J Pharmacol Exp Ther 2006;318:890–8.
[70] Roosevelt RW, Smith DC, Clough RW, Jensen RA, Browning RA. Increased extracellular concentrations of norepinephrine in cortex and hippocampus following vagus nerve stimulation in the rat. Brain Res 2006;1119:124–32.
[71] Hassert DL, Miyashita T, Williams CL. The effects of peripheral vagal nerve stimulation at a memory-modulating intensity on norepinephrine output in the basolateral amygdala. Behav Neurosci 2004;118:79–88.
[72] Manta S, Dong J, Debonnel G, Blier P. Optimization of vagus nerve stimulation parameters using the firing activity of serotonin neurons in the rat dorsal raphe. Eur Neuropsychopharmacol 2009;19:250–5.
[73] Snead 3rd OC. On the sacred disease: the neurochemistry of epilepsy. Int Rev Neurobiol 1983;24:93–180.
[74] Sorenson RL, Elde RP, Seybold V. Effect of norepinephrine on insulin, glucagon, and somatostatin secretion in isolated perifused rat islets. Diabetes 1979;28:899–904.
[75] Rulifson EJ, Kim SK, Nusse R. Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. Science 2002;296:1118–20.