3. Introduction

3.1. Small intestinal strangulation in horses

3.1.1. Incidence

Colic is a major cause of mortality in horses, representing the single most common cause of death (1). The incidence of colic in different populations of horses has been reported to lie between 3.5 to 10.6 colic episodes per 100 horses per year (2). Of the horses treated for colic in veterinary hospitals, 25% to 64% are affected with small intestinal disease (3-6). The majority of these cases (58% to 85%) are caused by strangulating lesions with concurrent intestinal ischaemia (4-7). The most common strangulating small intestinal lesions in horses are pedunculated lipomas, epiploic foramen entrapment, volvulus and intussusception (3, 8-10).

3.1.2. Pathogenesis

Small intestinal strangulation results in the obstruction of the lumen and the intestinal blood supply. These obstructions can be classified as haemorrhagic or ischaemic strangulating obstructions (11, 12). The first occurs when the veins are occluded more than the arteries, resulting in severe oedema and haemorrhage, also known as warm or low-flow ischaemia (11-14). In contrast, ischaemic strangulating obstructions evoke the complete occlusion of both arterial and venous blood supply, resulting in a pale appearance and rapid tissue degeneration (11, 12, 15, 16). This type of obstruction is also known as cold or no-flow ischaemia.

Intestinal injury occurs as a result of a reduction in oxygen supply. Small intestinal mucosa is known to be very susceptible to ischaemic injury due to its high energy demand and the hypoxic state of the villus tip even under normal conditions (17, 18). On a cellular level, the anaerobic metabolism increases and lactate accumulates, causing a decrease in ATP level and intracellular pH. Ion transport mechanisms become dysfunctional, resulting in calcium overload, cell swelling with subsequent rupture, and cell death by necrotic, necroptotic, apoptotic, and autophagic mechanisms (19). On a histopathological level, this results in the separation of the enterocytes from the lamina propria, inflammatory infiltration, apoptosis and cell necrosis (13, 20). Additionally, seromuscular injury may develop with mesothelial cell loss, leucocytic infiltration and oedema (21). Furthermore, in case of haemorrhagic strangulating obstructions, the resulting haemorrhage may disrupt the tissue architecture (22).

After resolution of the strangulating obstruction, intestinal tissue damage may continue due to reperfusion injury (23, 24). After reestablishment of the blood supply, xanthine oxidase rapidly degrades hypoxanthine thereby producing reactive oxygen species (25). These cause oxidative tissue damage and trigger the arachidonic acid metabolism to generate neutrophil chemo-attractants (26, 27). Even though inflammatory infiltration is considered a part of normal tissue repair, neutrophils can exacerbate tissue injury, and their infiltration during this

phase likely contributes to the occurrence of severe post-operative complications such as adhesions and paralytic ileus (12, 28-31). It has been suggested that no-flow ischaemia induces maximal injury during the ischaemic phase (12, 24, 32). Contrarily, experimental studies implementing low flow ischaemia models have demonstrated the occurrence of reperfusion injury in the equine small intestine (14, 32). The significance of reperfusion injury and its contribution to morbidity in clinical cases of strangulating obstruction remains a point of controversy.

3.1.3. Assessment of intestinal viability

Strangulating intestinal obstructions are generally treated by manual resolution of the lesion through a ventral midline laparotomy under general anaesthesia (11, 12). After resolution of the strangulation, the assessment of intestinal viability is of great importance to determine the necessity of small intestinal resection and for determining the prognosis. Colour, wall thickness, presence of peristalsis, and palpable mesenteric arterial pulsation can be used for clinical assessment of the intestine (33). However, changes in colour due to intramural haemorrhage and oedema can be misleading, and it was reported that clinical assessment was only 54% accurate in predicting viability (34). Several ancillary diagnostic methods for the assessment of intestinal lesions have been reviewed, including surface oximetry, Doppler ultrasonography and fluorescein dye (12, 35). However, these methods have not been proven reliable for all types of lesions and clinical application is limited (34). Histology is considered to be the gold standard for determining viability (36, 37). Nevertheless, it is not possible to perform this intra-operatively due to time constraints. Consequently, the clinical assessment remains the most important factor for decision making, even though the crucial factors for and against resection remain a point of discussion among surgeons (12).

3.1.4. Small intestinal resection – prognosis and limitations

If the intestine is judged to be non-viable, small intestinal resection followed by intestinal anastomosis needs to be performed. Survival rates after intestinal resection have increased in the past decades compared to earlier studies (38). Currently, around 80% of the horses survive until discharge, yet a trend towards lower survival rates is found after resection of more than 2 m of intestine (8, 39-41). Adhesions, post-operative ileus and endotoxaemia are common causes of nonsurvival after surgery for small intestinal strangulating obstructions (12). Horses that require small intestinal resection have a poorer short-term survival than horses that do not require resection (42, 43). It must also be noted that in some cases the complete resection of the affected segment may not be possible due to anatomic restrictions, for example in lesions that involve the duodenum or ileum (44). Furthermore, resecting of more than 60 - 75% of the total intestinal length may cause short bowel syndrome (11, 45, 46). Determining the exact site of resection can be precarious, as distension at the periphery of a strangulating lesion can induce injury that is difficult to detect, and resection margins may not be as healthy as would be expected based on the clinical judgement (16, 47). This is of significance, as it has

been shown that leaving nonviable small intestine in situ is associated with progressive ischaemic injury, a high rate of early repeat celiotomy and low survival rates (42, 48). Consequently, all these factors need to be considered and complicate the decision making in the surgical treatment of small intestinal strangulating lesions.

3.1.5. Adjunctive treatment strategies

As mentioned in the previous section, small intestinal resection cannot serve as the solution for all cases of strangulating obstructions. Moreover, assessment of intestinal viability has not been proven to be highly reliable, and grossly unaltered intestinal tissue may already have undergone irreversible damage only apparent at histological level. Therefore, alternative treatment strategies are necessary to ameliorate ischaemia reperfusion injury of the intestine during colic surgery. Although the clinical significance of reperfusion injury is inconclusive at this time, many pharmacological substances have been tested for their ability to attenuate oxidative damage after intestinal ischaemia. Experimental trials in equine small intestinal ischaemia models have investigated the administration of DMSO, Allopurinol, intraluminal oxygen and 21-Aminosteroid without detecting a reduction in mucosal injury (32, 49). In contrast, the local application of customized solutions containing combinations of different substances such as allopurinol, deferoxamine and adenosine did ameliorate mucosal and microvascular injury (50, 51). Superoxide dismutase, lidocaine, platelet-activating factor antagonist, high-molecular-weight dextrans, manganese chloride, and acetylcysteine have also been tested in experimental trials, with variable success (27, 52-55). Clinical trials are lacking, and except for the use of systemic lidocaine, these therapies have not found acceptance and implementation in clinical practice.

3.2. Preconditioning

As in veterinary medicine, ischaemic injury of different organs and tissues has a significant impact in human medicine. Especially ischaemic heart diseases cost many lives each year (56). They represent a major drive for the development of new treatment strategies to ameliorate myocardial ischaemia reperfusion injury. This is how the concept of preconditioning came into life. This phenomenon abides by the principle of hormesis which refers to the adaptive cellular responses after exposure to low doses of a noxious stimulus, increasing the tolerance of the cell or tissue to higher levels of a noxious stimulus (57).

3.2.1. Ischaemic preconditioning

Ischaemic or mechanical preconditioning was first discovered in 1986 by Murry et al. (58), who demonstrated that myocardial infarction in dogs was reduced by 75% when four cycles of 5 min coronary artery occlusion preceded 40 min of coronary ischaemia. Following this discovery, the phenomenon was extensively investigated in rodent, rabbit and canine models of myocardial ischaemia, demonstrating reduced infarct size and apoptosis rate, decreased

neutrophil accumulation, preserved vascular endothelial function and attenuation of the incidence and severity of post-ischaemic arrhythmias (59-63). This led to the implementation of ischaemic preconditioning by aortic clamping in human patients subjected to coronary artery bypass surgery. Reported positive effects were reductions in postoperative troponin release, improvements in the cardiac index, and a reduction in the need for postoperative inotropic support (64-66). Contrarily, other studies could not detect a positive effect on oxygen saturation, arterial pH, blood lactate, creatinine kinase and troponin release (67, 68).

The increasing popularity of IPC did not remain unnoticed in other medical fields; hence this concept was also adapted to other organs. For example, the cytoprotective effects have been demonstrated in the liver in several studies using a rat model (69, 70), and IPC was found to be an effective treatment against hepatic ischaemia in human patients undergoing hemihepatectomy (71).

In the field of small intestinal ischaemia, various experimental studies have been performed. The most commonly used experimental model is the occlusion of the cranial mesenteric artery (CMA) in rats, with significant variations in the time frame of the experiment. The duration of the main ischaemic event usually varied between 30 to 90 minutes, and the preconditioning ischaemic bouts ranged between 2 minutes and 1 hour, with 5 or 10 minutes being most commonly reported (72-78). Some authors who compared different IPC regimes demonstrated better protection with cycles of a shorter duration of 2 to 10 minutes (79, 80). It has also been shown that repeated episodes of ischaemia induce a better protective effect than a single ischaemic period (81). Another point of variation is the reperfusion time between preconditioning and main ischaemic episode, with a range between 5 minutes and 24 hours (72-78, 82).

These experimental studies have investigated many different aspects of intestinal ischaemia reperfusion injury. IPC was found to reduce the histomorphological changes in the intestinal mucosa (76, 78, 79, 83-87) and to induce less intestinal oedema with lower wet-to-dry ratios (77, 85). Lower apoptosis rates were reported, based on immunohistochemical staining for M30 (75, 76) or by Terminal deoxynucleotidyl transferase dUTP Nick-End Labeling (TUNEL) (72, 88). IPC was also associated with lower myeloperoxidase (MPO) activity as a marker for inflammation (76-78).

Evaluating oxidative stress variables, IPC reduced the generation of reactive oxygen species (88), and biochemical analysis of blood serum revealed decreased serum lactic dehydrogenase and lactate levels following IPC (83, 85, 86, 89). The intestinal tissue exhibited lower malondialdehyde levels and higher superoxide dismutase activity (72, 76-78). Furthermore, a lower percentage of xanthine dehydrogenase to xanthine oxidase conversion, uric acid concentration, and reduced glutathione were found in preconditioned rats (90). Looking at the effect on the barrier function of the mucosa, IPC ameliorated intestinal hyperpermeability (84) and prevented I/R-induced bacterial translocation (87). Other functional parameters that were improved by IPC include the transit of intestinal content (78), intestinal microvascular perfusion and tissue oxygenation (86), and reperfusion-induced hypotension (85). A study

investigating IPC in a pig model with occlusion of the CMA found similar results to those reported in the rat model (73).

Most studies investigated the short-term effects of IPC with reperfusion times limited to 30 to 120 minutes, with only few authors applying a longer duration of reperfusion. At 24 hours, IPC improved the mucosal perfusion and decreased the leukocyte-endothelial interactions, as shown by intravital fluorescence microscopy (91), and a similar positive effect on MPO levels and transit of intestinal content compared to the effect after 6 hours was shown (78). The effect of IPC on histomorphology at this time point was variable, with one group reporting a significant effect of IPC (91), while another could not confirm this (78).

Because IPC can be applied prior to organ transplantation, this treatment strategy has also been used in experimental models for small intestinal grafting with segments of jejunum. In rats, IPC decreased the mucosal histological damage and LDH, AST and ALT levels (92, 93). Other reported positive effects were lower MPO levels (93), a lower apoptotic index, and a lesser degree of epithelial basement membrane disruption, as shown by transmission electron microscopy (94). An experimental study in pigs found higher levels of GSH and SOD as well as decreased oxygen free radicals (95). The same research group reported similar results when it performed the same experiment in dogs (96).

One experimental study in horses found that IPC prevented the progression of histological injury during reperfusion, yet IPC was also associated with increased infiltration of inflammatory cells (97). To the author's knowledge, there are so far no reports of the implementation of intestinal IPC in clinical trials in any species.

3.2.2. Pharmacological preconditioning

Preconditioning with pharmacological agents can also ameliorate ischaemic injury. After the discovery of ischaemic preconditioning, a substantial number of studies were performed that followed the concept of pharmacological preconditioning (PPC). As with IPC, most studies were undertaken in experimental myocardial ischaemia models, and a wide range in pharmacological agents have been shown to induce intrinsic protective mechanisms against ischaemia and reperfusion injury (98, 99). The pharmacological substances that have been tested can be divided into two categories. Firstly, specific triggers and signalling molecules have been investigated to determine the mechanisms behind IPC. Furthermore, drugs that are used in clinical practice have been tested for their ability to mimic the effect of and possibly replace IPC.

The most commonly reported effects of intestinal IPC were also demonstrated after PPC. A variety of pharmacological substances were shown to reduce histomorphological injury, apoptosis, and biomarkers for oxidative stress and inflammation (76, 100-102). Examples of triggers or downstream signalling molecules tested for ameliorating intestinal injury are adenosine (76, 101), calcitonin gene-related peptide (100) and erythropoietin (103). PPC with Vitamin C also attenuated intestinal injury (104). Furthermore, in an attempt to elucidate how the protective effect of IPC is mediated, substances that specifically inhibit possible target

receptors have been extensively investigated. This topic will be reviewed in more detail in the section on the mechanism of action (chapter 3.5).

More relevant for direct translation to clinical cases are studies investigating the PPC effect of drugs used in clinical practice. Volatile anaesthetics such as isoflurane and sevoflurane exhibit a dose-dependent preconditioning effect against IR-induced renal and intestinal injury (101, 105). Pretreatment with morphine prior to ischaemia and reperfusion mimicked the protection induced by intestinal IPC (77). N-acetylcysteine preconditioning in pigs undergoing small intestinal transplantation reduced the systemic levels of inflammatory markers (106). Several studies have reported the PPC effect of the highly selective α2-adrenergic receptor agonist dexmedetomidine on intestinal injury in rabbits and rats (102, 107). Moreover, this protective effect could also be demonstrated in an experimental model of equine small intestinal strangulation (97, 108). Of the above mentioned drugs only the inhalation anaesthetics are routinely used in the anaesthetic management of horses. The administration of dexmedetomidine has also been proven safe and effective in horses (109); however, there are no commercially available formulations that are registered for horses. Xylazine and lidocaine, both routinely used in the management of small intestinal colic (110), have not been investigated for their ability to elicit PPC.

3.3. Postconditioning

3.3.1. Ischaemic postconditioning

Due to the unpredictable nature of most ischaemic events, the concept of preconditioning is of limited practicability as treatment for patients with acute ischaemic disease. Almost 20 years after the discovery of preconditioning, the principle of ischaemic postconditioning (IPoC) was introduced. This first report of IPoC demonstrated that gradual and controlled reperfusion was more effective than abrupt elimination of the vascular occlusion in salvaging the ischaemic myocardium in dogs (111). This builds on the hypothesis that the protective mechanisms exerted by preconditioning may be activated after the blood supply has been reinstated (112). Ischaemic postconditioning is defined as delayed reperfusion by the repeated re-occlusion of the relevant blood vessels directly after an ischaemic event (99, 111).

As with IPC, most studies were performed in the field of cardiology. Experimental trials investigating myocardial ischaemia in rats subjected to IPoC found a decrease in the size of myocardial infarcts and less extensive oedema, reduced occurrence of arrhythmias, lower oxidative stress levels, reduced MPO activity, and improved ion pump function (111, 113-115). This has led to the application of IPoC in human patients with acute myocardial infarction, where postconditioning can be applied after thrombolysis by reocclusion of the coronary artery with an intravascular balloon catheter (116, 117). A meta-analysis of clinical trials has identified beneficial effects of this treatment in some of the tested variables, reporting improvement in the myocardial salvage index and decreased myocardial oedema, yet without affecting final infarct size, left ventricular volume or microvascular obstruction (118).

A protective effect of IPoC has also been reported in experimental ischaemia of liver (119), kidney (120), brain (121), lung (122), spinal cord (123), and testis (124). Furthermore, a large set of experimental trials in the rat CMA model for small intestinal ischaemia have shown many beneficial effects in rats subjected to IPoC (72, 125-133). Comparable to the situation with IPC, it is a topic of discussion whether the efficacy of IPoC is primarily due to the number of cycles, the duration of each cycle, or the total duration of the IPoC algorithm. As with intestinal IPC, shorter and more frequent cycles of 5 - 10 seconds seem to confer better intestinal protection than re-occlusion cycles of longer duration (130, 131, 134). On the other hand, one study found no difference between 2 cycles of 2 minutes and 4 cycles of 30 secs (135). Regarding the timing of IPoC, it seems to be essential that it is initiated immediately after abolishment of ischaemia, since one study reported that the protective effects of IPoC were lost when it was first performed after 3 minutes (126).

Similar to the results of the intestinal IPC studies, IPoC was found to reduce histomorphological injury of the mucosa (72, 125-133), intestinal oedema (126, 132), and the amount of TUNEL positive apoptotic cells (72, 127-129). Furthermore, IPoC decreased the expression of cleaved caspase-3 and -9 (127, 128). Other signs of apoptosis and cell death such as DNA ladder formation after electrophoresis (72) and serum levels of intestinal-type fatty acid-binding protein (127) were also reduced by IPoC.

Expression of biomarkers for oxidative stress was significantly lower in the groups subjected to IPoC. This was reflected by lower plasma and tissue levels of MDA (72, 126, 128, 130), lactate (128, 132), LDH (127, 131), creatine kinase (CK) (130, 131), and diamine oxidase (DAO) (128). Furthermore, IPoC increased the levels of superoxide dismutase (SOD) (128) and caused less reduction in the concentration of the free thiol groups (131).

Inflammatory signs were found to be lower in the IPoC groups, reflected by lower plasma and tissue MPO levels (72, 126, 136). Moreover, plasma concentrations of tumour necrosis factor (TNF)-α and interleukin (IL)-6 were lower after IPoC (126, 131). Evaluating the mucosal barrier function after IPoC, one study reported reduced bacterial translocation and higher values for mean arterial pressure (137). Furthermore, IPoC has been shown to decrease the claudin-2 immunoreactivity in epithelial cells after ischaemia (137).

Only little research has been done in other animal models. Implementation of IPoC in a CMA occlusion model in mice resulted in decreased histomorphological injury and lower serum levels of intestinal-type fatty acid-binding protein, TNF-α and IL-6 (138, 139). One study that investigated segmental jejunal ischaemia in rabbits could not detect an influence of IPoC on the tested variables of mucosal histomorphology and intestinal wet-to-dry ratio (140). In a pig model for small bowel transplantation, IPoC reduced MDA concentration and increased glutathione and SOD levels (141). To the author's knowledge, no studies on the effect of IPoC have so far been performed in horses. Furthermore, no clinical reports of intestinal IPoC are available.

3.3.2. Pharmacological postconditioning

Comparable to PPC, pharmacological substances have also been found to mimic the effects of ischaemic conditioning when administered immediately after the ischaemic event. Most studies investigating pharmacological postconditioning (PPoC), have focused on mimicking or abolishing the effects of IPoC by stimulation or inhibition of certain triggers and receptors, to find out more about its mode of action. Many drugs that have shown a protective effect in PPC have also been tested for their PPoC ability, among them several anaesthetics that may be of clinical relevance in the horse. Post-ischaemic administration of sevoflurane has been shown to ameliorate mucosal injury and inflammation in pigs and rats (101, 142). PPoC with propofol alleviated pathological changes in intestines and lung, and decreased apoptosis and oxidative stress levels (143). One study that investigated dexmedetomidine PPC in horses also found decreased epithelial injury scores following dexmedetomidine PPoC (108).

An extensive discussion of all substances that have been investigated as PPoC mediator goes beyond the scope of this thesis and can be found in published reviews on this topic (98, 99, 144). Moreover, it must be noted that drugs eliciting a protective effect during reperfusion do not necessarily exert this protection through the mechanism of postconditioning in the sense of hormesis. Nonetheless, the increasing popularity of postconditioning has led to the widespread adaptation of this term for the administration of drugs following ischaemia.

3.4. Remote conditioning

Another treatment strategy that has been discovered in relation to ischaemic conditioning is remote conditioning (RC). This describes the phenomenon that the protective effects of IPC and IPoC are not limited to the organ directly exposed to the bouts of conditioning ischaemia (99). Such protection in organs distant to those that are preconditioned enables the initiation of cell survival programmes in tissues that cannot be preconditioned directly in clinical situations. Remote conditioning has been shown to elicit a protective effect when performed prior to the main ischaemic event (remote preconditioning)(145), or directly after ischaemia (remote postconditioning) (146). This concept has been explored in different organs, mainly applying upper or lower limb ischaemia with a tourniquet as remote conditioning stimulus (145, 147-149). Alternatively, intraoperative occlusion of the infrarenal aorta, the renal artery or the hepatic pedicle have been performed (146, 150, 151). Experimental studies have reported attenuated ischaemia reperfusion injury following remote conditioning in different organs including the heart (146), the brain (147), the spinal cord (148), the kidney (151), and the small intestine (149, 150).

Remote conditioning has been applied to clinical cases, mainly human patients undergoing cardiac or renal surgey, or suffering from acute cerebral infarction (152-155). Resulting clinical trials have reported variable beneficial effects (152-155). In clinical cases of kidney transplantation, remote preconditioning was feasible and safe, yet no benefit in terms of the clinical outcome was observed (156). Remote postconditioning after acute cerebral infarction

was associated with improvement in some of the tested variables, including stroke scores and several biomarkers (157). In experimental intestinal ischaemia RC significantly reduced histological damage, inflammation and oxidative stress in the rat CMA model (158, 159). Lower limb ischaemia can also cause remote injury to the gastrointestinal system, and remote postconditioning can have a protective effect on this type of injury, with reduced histological damage and increased intestinal blood flow (150) as well as reduced expression of biomarkers for inflammation and oxidative stress (149). An experimental study looking at small intestinal anastomosis healing could not detect a beneficial effect of remote preconditioning (160). There are no reports on the application of RC in clinical patients with intestinal ischaemia.

3.5. Conditioning – the mechanism of action

Many studies in different tissues and experimental models have shown a protective effect of pre-, post- or remote conditioning. Similarly, a great amount of research has been dedicated to find the protective mechanism behind this phenomenon. Even though there may be differences between the different strategies, tissues and species, many of the identified triggers and pathways seem to play a role in all of them. It has been suggested that these pathways are activated during early reperfusion in both IPC and IPoC, possibly explaining the similar mechanism of action despite the difference in timing (112). The protective effects can be divided into an early phase of protection which induces the activation of pre-existing effectors, and a late phase of protection mediated by the upregulation of cytoprotective proteins (Fig.1) (99, 147, 161, 162).

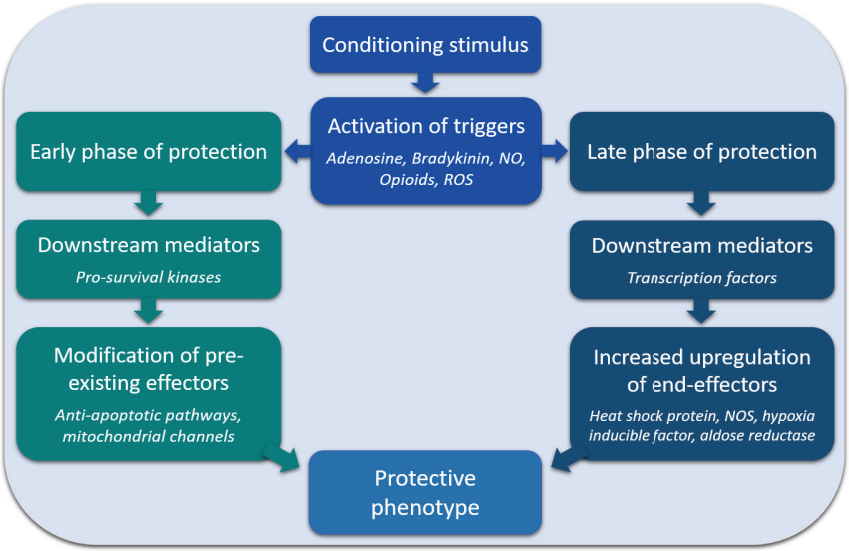

Figure 1: Diagram illustrating the proposed mechanism of action of conditioning (99, 147, 161, 162).

Using models of acute myocardial and intestinal ischaemia it has been shown that the protective effects of pre- and postconditioning are mediated by the activation of adenosine (76, 84, 163-165), bradykinin (166, 167), and opioid receptors (77, 168-170). The next step in the conditioning signalling cascade is the activation of pro-survival kinases such as PI3K/Akt and ERK-1/2, (112, 171, 172). With regard to intestinal postconditioning, the activation of Akt (133, 139), Nrf2 (133) and the JAK/STAT pathway (136) have been identified. These pro-survival pathways may initiate the expression of specific micro-RNAs (miR) and circular RNAs for subsequent regulation of protein expression (138). Several studies have found increased expression of miR-21 in response to IPC and IPoC (129, 172, 173). Another possible signalling molecule is nitric oxide. There are several studies that detected an increase in the synthesis of nitric oxide after ischaemic conditioning, and found that inhibition of nitric oxide synthesis abolished the protective effect of IPC (87, 89, 93).

A potential signalling protein that has received a lot of attention in the last few years is hypoxia inducible factor 1-alpha (HIF-1α). HIF-1α mediates many effects of hypoxia by acting as transcription factor (174, 175). HIF-dependent regulation of claudin-1 plays an important role in maintenance of intestinal epithelial tight junction integrity (176). Moreover, it has been shown that HIF-1α expression increases in ischaemic intestinal mucosa (177). The expression of the isoform HIF-2α is also increased in different tissues including the intestine under hypoxic conditions (178). However, HIF-2α has so far not been investigated in intestinal ischaemia. It has been suggested that HIF-1α plays a signalling role in the protective mechanism of IPoC. In experimental models of intestinal and myocardial ischaemia, HIF-1α expression was higher in groups subjected to IPoC compared to the untreated control groups, and was accompanied by less tissue damage (129, 179, 180). Remote conditioning and PPoC have also found to be associated with increased HIF-1α levels (154, 158). There is some evidence that HIF-1α exerts its protective effect through upregulation of miR-21 (129, 180). Contrarily, other investigations on IPC and IPoC in different experimental models have found an association between HIF-1α levels and the extent of tissue injury (181-183). In horses, it was found that manipulated intestinal tissue exhibited lower HIF-1α levels compared to control tissue (184). It has been suggested that the duration and severity of the I/R insult dictate whether HIF-1α plays a deleterious or a protective role (185). Hence, it remains unclear if HIF-1α represents a marker for the protective response, for the degree of I/R injury, or for both.

A great variety of downstream targets have been suggested to play a role in the protective mechanism of conditioning (99, 144). One of the main targets is the attenuation of apoptosis by modulating the expression of regulating factors such as *BCL-2* protein, as shown in both myocardial and intestinal IPoC (75, 170, 172). Increased expression of LC3 II/I, Beclin-1, and p62 was shown to mediate an IPoC-dependent increase in autophagy (133). Furthermore, the regulation of the mitochondrial permeability transition pores (mPTP) and the mitochondrial K_{ATP} channels appears to represent a common end effector (85, 88, 186-188).

The upregulation of protective proteins has also been suggested to be of importance,