Effects of NF-κB inhibition on mesenteric ischemia-reperfusion injury

Lei Zou,1,3 Bashir Attuwaybi,2,3 and Bruce C. Kone1,3

1Departments of Internal Medicine and of Integrative Biology and Pharmacology, 2Department of Surgery, and 3Trauma Research Center, The University of Texas Medical School at Houston, Houston, Texas 77030

Submitted 10 October 2002; accepted in final form 2 December 2002

Zou, Lei, Bashir Attuwaybi, and Bruce C. Kone. Effects of NK-κB inhibition on mesenteric ischemia-reperfusion injury. Am J Physiol Gastrointest Liver Physiol 284: G713–G721, 2003. First published December 4, 2002; 10.1152/ajpgi.00431.2002.—Mesenteric ischemia-reperfusion injury is a serious complication of shock. Because activation of nuclear factor-κB (NF-κB) has been implicated in this process, we treated rats with vehicle or the IκB-α inhibitor BAY 11-7085 (25 mg/kg ip) 1 h before mesenteric ischemia-reperfusion (45 min of ischemia followed by reperfusion at 30 min or 6 h) and examined the ileal injury response. Vehicle-treated rats subjected to ischemia-reperfusion exhibited severe mucosal injury, increased myeloperoxidase (MPO) activity, increased expression of interleukin-6 and intercellular adhesion molecule 1 protein, and a biphasic peak of NF-κB DNA-binding activity during the 30-min and 6-h reperfusion courses. In contrast, BAY 11-7085-pretreated rats subjected to ischemia-reperfusion exhibited less histological injury and less interleukin-6 and intercellular adhesion molecule 1 protein expression at 30 min of reperfusion but more histological injury at 6 h of reperfusion than vehicle-treated rats subjected to ischemia-reperfusion. Studies with phosphorylation site-specific antibodies demonstrated that IκB-α phosphorylation at Ser32,Ser36 was induced at 30 min of reperfusion, whereas tyrosine phosphorylation of IκB-α was induced at 6 h of reperfusion. BAY 11-7085 inhibited the former, but not the latter, phosphorylation pathway, whereas α-melanocyte-stimulating hormone, which is effective in limiting late ischemia-reperfusion injury to the intestine, inhibited tyrosine phosphorylation of IκB-α. Thus NF-κB appears to play an important role in the generation and resolution of intestinal ischemia-reperfusion injury through different activation pathways.

ileum; inflammation; ischemic bowel; transcription factor; ileus

Address for reprint requests and other correspondence: B. C. Kone, Depts. of Internal Medicine and of Integrative Biology and Pharmacology, The University of Texas Medical School at Houston, 6451 Fannin, MSB 4.138, Houston, TX 77030 (E-mail: Bruce.C.Kone @uth.tmc.edu).

MESENTERIC ISCHEMIA-REPERFUSION is an often-fatal clinical problem that complicates septic, cardiogenic, and hemorrhagic shock, vascular surgery, and small bowel transplantation. Mesenteric ischemia-reperfusion causes histological evidence of mucosal injury and gut dysfunction characterized by increased intestinal epithelial permeability and impaired motility (12). Ischemia-reperfusion injury evokes a molecular and cellular inflammatory response within the intestine, including activation of the transcription factors nuclear factor-κB (NF-κB) and signal transducer and activator of transcription 3, induction of granulocyte colony-stimulating factor and interleukin (IL)-6, and recruitment of neutrophils and monocytes into the intestinal muscularis (12, 15, 19, 31, 34, 38). Several molecular mediators have been implicated in mesenteric ischemia-reperfusion injury, including cytokines (6), reactive oxygen species (10), nitric oxide (33), arachidonic acid derivatives (26), and cell adhesion molecules (27).

NF-κB is known to be activated in the gut by a number of proinflammatory stimuli, including sepsis (7), cytokines (29), and oxidative stress (3). NF-κB activation leads to the coordinated expression of many genes that encode proteins involved in mediator synthesis and the further amplification and perpetuation of the inflammatory response, including proinflammatory cytokines, adhesion molecules, chemokines, growth factors, and inducible enzymes such as cyclooxygenase-2 and inducible nitric oxide synthase (9). Consequently, NF-κB is an obvious target for anti-inflammatory treatment. Pharmacological agents that inhibit NF-κB include glucocorticoids (1), antioxidants (37), certain cyclooxygenase inhibitors (35), proteasome and calpain inhibitors (23), and inhibitors of IκB-α phosphorylation (28).

NF-κB is usually kept inactive in the cytoplasm through association with a member of the IκB family. The most well-characterized pathway of NF-κB activation involves phosphorylation of IκB-α on Ser32,Ser36 by the IκB kinase complex. This phosphorylation event targets IκB-α for ubiquitination and degradation by the ubiquitin-26S proteasome pathway, allowing nuclear transmigration of NF-κB, where the transcription factor binds to specific DNA sequences located in the promoter regions of many proinflammatory genes (9, 18, 30, 36). A wide array of stimuli, including cytokines, endotoxin, and phorbol esters, activates this IκB-α phosphorylation-ubiquitination-degradation pathway. Two additional activation pathways have been reported. One results from oxidant stress (21, 25, 32) or perversely treatment (17) and appears to require phosphorylation of IκB-α at Tyr42. The dissocia-

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
tion of tyrosine-phosphorylated IκB-α from NF-κB is not the result of degradation by the 26S proteasome (17). This pathway appears to be specific for IκB-α, because Tyr42 is not conserved in other IκB family members. The exact protein tyrosine kinase(s) or phosphatase(s) involved in this pathway remains unknown, but members of the Src family have been implicated. Moreover, this activation pathway is well characterized only in lymphocytes, and there are no data for the ileum in vivo. The second atypical scheme for NF-κB activation occurs in cells exposed to short-wavelength ultraviolet light and involves induction of IκB-α degradation by the 26S proteasome, without phosphorylation of Ser32, Ser36, or Tyr42 (4, 24). The mechanisms for this alternative pathway of IκB-α degradation are unknown. Both of these alternative pathways yield lower levels of NF-κB activation than have been observed in response to classical activators such as cytokines or endotoxin.

Work from our laboratory (13) and others (15, 38) in experimental animal models has established that NF-κB is activated in the small intestine after mesenteric ischemia-reperfusion. We showed that mesenteric ischemia-reperfusion results in a biphasic activation pattern of NF-κB DNA-binding activity in the post-ischemic rat ileum during reperfusion. After 45 min of mesenteric ischemia, the first peak of NF-κB DNA-binding activity occurred at 30 min of reperfusion, followed by lower levels of activity and a second peak after 6 h of reperfusion. We further demonstrated that exogenous administration of the anti-inflammatoryneuropeptide α-melanocyte-stimulating hormone (α-MSH) before mesenteric ischemia-reperfusion protected intestinal transit and morphology and reduced ischemia-reperfusion-induced activation of intestinal NF-κB after prolonged (6 h) reperfusion (13). This association of reduced late NF-κB activity and protection against mesenteric ischemia-reperfusion injury suggested that targeted inhibition of NF-κB at the onset of mesenteric ischemia-reperfusion might prove protective. Accordingly, in the present report, we tested the in vivo effects of [(E)-3-(4-t-butylphenylisulfonyl)-2-propenenitrile] (BAY 11-7085), a compound that inhibits NF-κB activation by blocking inducible IκB-α phosphorylation at Ser32, Ser36 (28), on mesenteric ischemia-reperfusion injury, NF-κB activation, MPO activity as an index of neutrophil influx, and expression of cytokine and adhesion molecule genes known to be upregulated in intestinal ischemia-reperfusion injury. This compound has been shown to inhibit NF-κB activation and to exert anti-inflammatory activity in vivo in several models of inflammation (20, 22, 28). Our results indicate a dual effect of the NF-κB inhibitor to abrogate early intestinal ischemia-reperfusion injury but exacerbate ischemia-reperfusion injury at later stages of reperfusion. These distinct phases coincide with NF-κB activation through different IκB-α phosphorylation pathways. The data suggest that NF-κB may play a role not only in the initiation of mesenteric ischemia-reperfusion injury but also in a late repara-tive process that at least partly depends on the specific mechanisms of IκB-α phosphorylation.

**MATERIALS AND METHODS**

**Animal model.** Male Sprague-Dawley rats (Harlan Labs, Houston, TX; 250–350 g body wt) were cared for in accordance with the guidelines of The University of Texas Medical School at Houston Animal Welfare Committee. The rats were fed standard rat chow and water ad libitum. Operative procedures were performed using standard sterile technique under general anesthesia with inhaled isoflurane. The animals were fasted for 18 h before the operative procedures. The procedures for superior mesenteric artery (SMA) occlusion (45 min) and sham surgery were as previously described for our laboratory (13). BAY 11-7085 (25 mg/kg; Biomol) or an equal volume of vehicle (polyethylene glycol 400 diluted 1:5 in 5% bovine serum albumin/H2O) was administered intraperitoneally before the SMA was clamped. This concentration of BAY 11-7085 has been shown to be effective in other in vivo models of inflammation (20, 22, 28). The rats were killed 30 min or 6 h after release of the SMA clamp or sham surgery. Histology, NF-κB DNA-binding activity, ICAM-1 and IL-6 expression, MPO activity, and immunoblots or immunoprecipitations for phosphorylated IκB-α at Ser32, Ser36 or tyrosine residues were then determined. Thus the following groups of animals were studied: sham surgery with ischemia followed by reperfusion at 30 min [sham + I/R (30 min)], vehicle with ischemia followed by reperfusion at 30 min [Veh + I/R (30 min)], BAY 11-7085 treatment with ischemia followed by reperfusion at 30 min [BAY + I/R (30 min)], vehicle with ischemia followed by reperfusion at 6 h [Veh + I/R (6 h)], and BAY 11-7085 treatment with ischemia followed by reperfusion at 6 h [BAY + I/R (6 h)].

**Histological assessment of injury.** In a separate set of experiments, biopsies of the distal ileum were taken from sham-operated animals and animals subjected to mesenteric ischemia-reperfusion treated with vehicle or BAY 11-7085 after 45 min of SMA occlusion and 30 min or 6 h of reperfusion. Tissues were fixed with 10% neutral-buffered formalin and processed by routine techniques before they were embedded in paraffin wax. Sections (4 μm) were stained with hematoxylin and eosin and examined under a light microscope. For histological assessment of intestinal injury, we graded the injury on a scale of 0–4, where 0 is normal histology, 1 is slight disruption of the surface epithelium, 2 is epithelial cell loss injury at villus tip, 3 is mucosal vascongestion, hemorrhage, and focal necrosis with loss of less than one-half of villi, and 4 is damage extending to more than one-half of villi. All processed tissues were examined under light microscopy by two blinded, experienced observers, and the scores of the observers were averaged.

**Electrophoretic mobility shift and supershift assays.** Nuclear and cytoplasmic extracts from full-thickness ileal tissue were prepared by the method of Deryckere and Gannon (8) as described previously (13). The DNA-binding activity of NF-κB in ileal nuclear extracts was determined by electrophoretic mobility shift assay (EMSA). The NF-κB consensus oligonucleotide 5′-AGT TGA GGC GAT CCC AGG C-3′ (Promega, Madison, WI) was end labeled with [γ-32P]ATP using T4 polynucleotide kinase. Nuclear extract (10 μg) was then incubated for 20 min with gel shift binding buffer [10 mM Tris, pH 7.5, 50 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 5% glycerol, and 1 μg poly(dI-dC)] and 1 μl of labeled probe. For competition assays, a 100-fold molar excess of unlabeled NF-κB oligonucleotide was added to the binding reaction. For supershift assays, 2 μl of antibody to NF-κB...
subunit p50 or p65 (Santa Cruz Biotechnology, Santa Cruz, CA) were added before addition of the labeled probe. Gel loading buffer was added to the mixture, and the samples were electrophoresed on a nondenaturing 5% polyacrylamide gel. The gels were then dried and analyzed by autoradiography.

Western blotting and immunoprecipitation. For Western blots, cytoplasmic extracts were directly resuspended in SDS sample buffer, boiled for 5 min, and analyzed on 7.5% or 10% polyacrylamide gels as described previously. The proteins were electrophoretically transferred to polyvinylidene difluoride membranes (Hybond ECL, Amersham) and probed with antibodies against IL-6, ICAM-1, IkB-α, or Ser32,36-phosphorylated IkB-α (all from Santa Cruz Biotechnology) by methods routinely used in our laboratory (39). Protein blots of the immunoprecipitates were probed with anti-IkB-α antibodies, and processed for enhanced chemiluminescence detection as described above.

MPO assay. Cytoplasmic extracts from full-thickness ileal tissue were diluted 1:5 in buffer A (0.6% Nonidet P-40, 150 mM NaCl, 10 mM HEPES, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, and 30 μl/ml protease inhibitor cocktail). Ten microliters of each sample were then added to wells of 96-well plates and incubated with 100 μl of tetramethylbenzidine peroxidase substrate (KPL, Gaithersburg, MD) at room temperature for 20 min. The reaction was stopped with 100 μl of 0.18 M sulfuric acid. Optical density was measured at 450 nm with an ELISA plate reader. Assays were performed in duplicate, and the results were normalized for protein content.

Statistical analysis. Quantitative data are expressed as means ± SE and were analyzed with one-way analysis of variance (ANOVA) or t-test as appropriate. P < 0.05 was considered significant.

RESULTS

BAY 11-7085 protects against early, but exacerbates late, mesenteric ischemia-reperfusion injury in ileum. Representative hematoxylin-and-eosin-stained sections of ileum from Veh + I/R (30 min), Veh + I/R (6 h), BAY + I/R (30 min), and BAY + I/R (6 h) rats are depicted in Fig. 1A. The degree of ischemia-reperfusion-induced mucosal damage expressed as a microscopic damage score is shown in Fig. 1B. In the sham-operated group, microscopic assessment of the tissue revealed no significant abnormalities. In agreement with our earlier report (13), mesenteric ischemia-reperfusion caused significant epithelial abnormalities as well as infiltration of inflammatory cells into the villi (Fig. 1). Pretreatment with BAY 11-7085 compared with vehicle + mesenteric ischemia-reperfusion reduced the ischemia-reperfusion-associated increase in the microscopic damage score at 30 min of reperfusion (Fig. 1). At 6 h of reperfusion, vehicle-treated mesenteric rats subjected to ischemia-reperfusion exhibited more prominent neutrophil infiltration and capillary congestion, but injury was significantly less than in Veh + I/R (30 min) rats (Fig. 1). In BAY + I/R (6 h) rats, these signs of tissue injury and inflammation were much more severe (Fig. 1). Thus NF-κB inhibition by BAY 11-7085 was beneficial in the initial response to ischemia-reperfusion injury but exacerbated injury at later phases of reperfusion.

BAY 11-7085 inhibits early IL-6 and ICAM-1 expression in ileum after mesenteric ischemia-reperfusion. IL-6 is known to be induced in the gut early in the postischemic phase and to participate in the inflammatory response (15). ICAM-1 is an adhesion molecule that is induced by various inflammatory mediators and plays an important role in gut ischemia-reperfusion-induced mucosal dysfunction and neutrophil infiltration (5, 34). Both of these proteins are regulated in part by NF-kB. To determine whether BAY 11-7085 inhibition of NF-κB modulated the expression of these molecules, immunoblot analysis of IL-6 and ICAM-1 expression in cytoplasmic extracts from full-thickness ileal tissues was performed in the various groups of animals. IL-6 was induced nearly twofold after mesenteric ischemia and 30 min of reperfusion but returned to its low basal level by 6 h of reperfusion (Fig. 2A and B). BAY 11-7085 pretreatment resulted in IL-6 expression levels approximating that of the sham controls at 30 min and 6 h of reperfusion (Fig. 2, A and B). ICAM-1 protein was induced in the Veh + I/R (30 min) rats to levels ~30% greater than in sham controls and remained elevated at 6 h of reperfusion compared with sham (Fig. 2, C and D). BAY 11-7085 completely inhibited the ischemia-reperfusion-induced expression of ICAM-1 at 30 min, but not at 6 h, of reperfusion (Fig. 2, C and D).

BAY 11-7085 inhibits early, but not late, induction of ileal MPO activity after mesenteric ischemia-reperfusion. Neutrophil infiltration occurs early in the course of mesenteric ischemia-reperfusion injury. To assess neutrophil infiltration, MPO activity in ileal homogenates was measured. Our previous study showed that MPO activity in ileal samples harvested from rats subjected to mesenteric ischemia and 6 h of reperfusion was significantly increased compared with that in samples from sham controls (13). BAY 11-7085 pretreated rats subjected to ischemia-reperfusion exhibited lower levels of MPO activity at 30 min, but not at 6 h, of reperfusion than vehicle-treated rats subjected to ischemia-reperfusion at the same time points (Fig. 3). There was a trend toward greater MPO activity in BAY + I/R (6 h) than in Veh + I/R (6 h) animals, but this did not achieve statistical significance. These MPO data are consistent with the effects of BAY 11-7085 on ICAM-1 expression after mesenteric ischemia-reperfusion (Fig. 2).

BAY 11-7085 inhibits early loss of IkB-α and induction of NF-κB activity in ileum after mesenteric ischemia-reperfusion. In vivo studies have demonstrated that BAY 11-7085 inhibits NF-κB-activated expression of ICAM-1, vascular cell adhesion molecule 1, E-selectin, IL-6, and IL-8, with IC50 values of 5–10 μM (28).
Inhibition of IκB-α phosphorylation and cell adhesion molecule expression and stabilization of IκB-α were irreversible in these studies (28). As an inhibitor of IκB-α, BAY 11-7085 would be expected to inhibit NF-κB DNA-binding activity in tissues exposed to the agent. To determine whether this occurred in the postischemic ileum, IκB-α protein abundance was measured by Western blot analysis of cytosolic extracts, and NF-κB DNA-binding activity was measured by EMSA in nuclear extracts prepared from sham-operated rats, as well as vehicle- and BAY 11-7085-pretreated rats subjected to mesenteric ischemia and 30 min or 6 h of reperfusion. IκB-α was constitutively expressed in ileal cytoplasmic samples from sham-operated rats (Fig. 4). A rapid loss of IκB-α from ileal cytoplasm occurred within 30 min of reperfusion but was present at near-initial levels at 6 h of reperfusion. In rats subjected to mesenteric ischemia-reperfusion, pretreatment with BAY 11-7085 effectively inhibited the loss of IκB-α at 30 min of reperfusion but insignificantly inhibited the loss of IκB-α at 6 h of reperfusion (Fig. 4).

Consistent with our previous observations (13), NF-κB DNA-binding activity in ileal nuclear extracts presented as two sequence-specific gel shift complexes (complex I and complex II) in all groups. Higher expression of κB-specific complex II was induced in vehicle-treated rats subjected to ischemia-reperfusion than in sham-operated rats, which exhibited a small amount of NF-κB DNA-binding activity (Fig. 5) at 30 min and 6 h of reperfusion. Nuclear NF-κB DNA complex II-binding activity was ~40% lower in BAY 11-7085-treated rats subjected to ischemia and 30 min of reper-
fusion but only showed a trend toward lower binding activity at 6 h of reperfusion than in vehicle-treated animals subjected to mesenteric ischemia-reperfusion at identical time points of reperfusion. Levels of IκB-α specific complex I were much lower than levels of complex II (Fig. 5A) and, although induced by ~25% compared with sham, did not differ significantly from vehicle-treated rats subjected to ischemia-reperfusion after BAY 11-7085 treatment (Fig. 5C). Thus, at 30 min of ischemia-reperfusion, IκB-α degradation was associated with NF-κB activation (predominantly complex II), and BAY 11-7085 inhibited both of these processes. In the later phase (6 h) of ischemia-reperfusion, NF-κB activity was induced, despite the fact that IκB-α degradation was unchanged, and BAY 11-7085 did not significantly inhibit NF-κB DNA-binding activity. This result suggested that an alternative mechanism for
Fig. 5. BAY 11-7085 inhibits activation of nuclear factor-κB (NF-κB) DNA binding activity in ileum early, but not late, after mesenteric ischemia-reperfusion injury. Rats were treated as described in Fig. 1 legend. Ileal samples were obtained, and nuclear extracts were prepared. A: electrophoretic mobility shift assay with an NF-κB consensus binding site probe. I and II, κB-specific DNA-protein complexes I and II (n = 3 for each group). Photograph has been cropped to exclude free probe. B: densitometric analysis of bands representing NF-κB-specific protein-DNA complex II from ileum of rats (n = 4 in each group). Values are means ± SE. C: densitometric analysis of bands representing NF-κB-specific protein-DNA complex I from ileum of rats (n = 4 in each group). Values are means ± SE. *P < 0.05 vs. Veh + I/R (30 min). D: nuclear extracts were prepared from ileal samples (n = 3 for each group) and subjected to supershift (SS) assays with an NF-κB consensus binding site probe and antibody against NF-κB p50 or p65.
NF-κB activation, not involving Ser\(^{32}\), Ser\(^{36}\) phosphorylation of IκB-α, might be operative in the later phases of ischemia-reperfusion.

Supershift experiments revealed the same pattern in all groups, with anti-p50 supershifting NF-κB-DNA complex II and anti-p65 partially supershifting NF-κB-DNA complex II (Fig. 5D). Thus NF-κB p50/p65 heterodimers appear to be induced in the nucleus of the ileum after mesenteric ischemia-reperfusion, in agreement with our previous work (13), and although it inhibits NF-κB DNA-binding activity, BAY 11-7085 pretreatment does not alter the protein composition of these DNA-protein complexes.

Differential responses of BAY 11-7085 and α-MSH in IκB-α phosphorylation pathways. On the basis of our findings in the present report that BAY 11-7085 inhibited IκB-α loss and NF-κB activation at 30 min, but not at 6 h of reperfusion and our earlier report that α-MSH inhibited NF-κB activity induced at 6 h of reperfusion (13), we hypothesized that the two agents might be acting on different signaling cascades that converge on NF-κB activation. Accordingly, using cytosolic extracts of ileum from rats subjected to ischemia-reperfusion, we studied IκB-α phosphorylation at Ser\(^{32}\), Ser\(^{36}\) by Western blot analysis with antibodies raised against IκB-α phosphorylated at these residues, analyzed tyrosine phosphorylation of IκB-α by immunoprecipitation with an antiphosphotyrosine antibody, and analyzed the immunoprecipitated proteins with anti-IκB-α by Western blot. IκB-α phosphorylation at Ser\(^{32}\), Ser\(^{36}\) was strongly induced at 30 min, but not at 6 h of reperfusion (Fig. 6A), consistent with our data concerning IκB-α degradation and NF-κB activation. In contrast, rats pretreated with BAY 11-7085 exhibited no increase in immunoreactivity for IκB-α phosphorylation at Ser\(^{32}\), Ser\(^{36}\) induction at either time point (Fig. 6A). Immunoreactivity for tyrosine-phosphorylated IκB-α was detected principally at 6 h of reperfusion; little immunoreactivity was detected at 30 min of reperfusion (Fig. 6B). BAY 11-7085 pretreatment had no inhibitory effect on tyrosine phosphorylation of IκB-α, as would be expected from the mechanism of action of the drug, at either time point (Fig. 6B). We also detected expression of tyrosine-phosphorylated IκB-α in ileum of sham animals, suggesting that this pathway may in part be responsible for the constitutive expression of NF-κB that has been observed by us and others.

Because we previously showed that α-MSH pretreatment inhibited late ileal NF-κB activation and injury in this ischemia-reperfusion model, in contrast to our present findings with BAY 11-7085, we tested the hypothesis that α-MSH acted by inhibiting tyrosine phosphorylation of IκB-α and, consequently, NF-κB activation in the late phase of ischemia-reperfusion. Indeed, whereas BAY 11-7085 had no inhibitory effect on tyrosine phosphorylation of IκB-α at 6 h of reperfusion, α-MSH partially inhibited the induction of immunoreactivity for tyrosine-phosphorylated IκB-α at this time point (Fig. 6B). Thus tyrosine phosphorylation of IκB-α and consequent activation of NF-κB are late events in ileal ischemia-reperfusion injury and are inhibited by α-MSH, but not by BAY 11-7085.

DISCUSSION

We used BAY 11-7085, an irreversible inhibitor of IκB-α phosphorylation on Ser\(^{32}\), Ser\(^{36}\), to test in vivo the hypotheses that signaling through NF-κB mediates, at least in part, mesenteric ischemia-reperfusion injury in the rat and that blockade of NF-κB activation would abrogate posts ischemic gut injury. In the aggregate, our data suggest that NF-κB activation by the classical pathway of serine phosphorylation of IκB-α plays an important role in the injury response of the ileum during the early reperfusion period following mesenteric ischemia. In addition, induction of this transcription factor via tyrosine phosphorylation of IκB-α appears to play a role in sustaining or aggravating injury cascades and/or blocking inflammatory resolution at later posts ischemic time points, because inhibition of this pathway by α-MSH (Fig. 6) also protected against late posts ischemic injury (13). Thus NF-κB activation, mediated by different phosphoryla-
tion pathways, is associated with the onset and aggravation or perpetuation of postischemic injury in this model. These results contrast with the reciprocal roles of NF-κB postulated in other in vivo studies of the effects of NF-κB inhibition on inflammation. In rat carrageenin pleurisy and mouse carrageenin air pouch models, NF-κB activation was associated with the onset and resolution of inflammation (22). During resolution, however, NF-κB activity was not associated with the release of proinflammatory mediators but rather with the expression of endogenous anti-inflammatory pathways and leukocyte apoptosis. Inhibition of NF-κB during the resolution of inflammation prolonged the inflammatory response, prevented clearance of leukocytes, and aggravated injury (22). The mechanistic basis for the different phases of NF-κB activation and, specifically, of IκB-α phosphorylation pathways in these models, however, was not established. Our data suggest that differential signaling via distinct phosphorylation events to NF-κB may underlie specific phases of postischemic injury and repair in the intestine.

Other factors may be responsible for the fact that IκB-α loss was much less at the later stage of reperfusion. It may be that ischemia-reperfusion in the later phase (6 h) of reperfusion disrupts the ATP-dependent ubiquitin-dependent proteosome pathway, which degrades phosphorylated IκB-α, so that any IκB-α that escapes inhibition by BAY 11-7085 and is phosphorylated would tend to accumulate. Indeed, ATP-dependent reassociation of the 20S catalytic and PA700 regulatory subunits to form the active 26S proteasome is severely, specifically, and irreversibly impaired in the hippocampus after transient forebrain ischemia (2). These possibilities and others require further investigation.

The detrimental effects of BAY 11-7085 treatment on postischemic injury at 6 h of reperfusion contrast with the protective effect of α-MSH on mesenteric ischemia-reperfusion injury at this time point of reperfusion we previously reported (13). Although BAY 11-7085 inhibited NF-κB activation more significantly early than late in the reperfusion period, α-MSH did the opposite, inhibiting late, but not early, NF-κB activation. Similarly, BAY 11-7085 inhibited early, but not late, MPO induction, whereas α-MSH inhibited late, but not early, MPO induction (early time points were not assessed in that study). The fact that α-MSH blocked only late tyrosine phosphorylation of IκB-α, whereas BAY 11-7085 inhibited early Ser32, Ser36 phosphorylation of IκB-α, provides a mechanistic basis for these discrepant results. However, because α-MSH exerts many other effects though signaling pathways other than NF-κB, other mechanisms could be operative. Our results showing that α-MSH did not alter IκB-α degradation rates are in agreement with studies in two cutaneous melanoma cell lines where the neuropeptide was found to inhibit NF-κB activation without affecting IκB-α protein levels (14). However, work in glioma cells and pulmonary epithelial cells (16) suggested that α-MSH inhibited IκB-α degradation and, consequently, NF-κB activation. Indeed, we previously demonstrated cell type-specific mechanisms by which α-MSH inhibits transcriptional activation of proinflammatory genes (11).

As in most cases of pharmacological inhibition, NF-κB inhibitors may have some nonspecific effects independent of the NF-κB pathway. However, the use of inhibitors allows modulation of NF-κB at specific stages of the inflammatory response in vivo, which is central to the findings reported here. Unfortunately, however, available molecular tools do not allow modulation of the NF-κB pathway at different stages of the inflammatory response in the gut in vivo. The present study indicates that the course of mesenteric ischemia-reperfusion injury in this model can be modulated by pharmacological interference with NF-κB activation, and that the therapeutic impact of such inhibition on the gut critically depends on the duration of reperfusion and the specific signaling cascades to NF-κB activation. These results also lead to the prediction that combined treatment with BAY 11-7085 and α-MSH would block both phases of NF-κB activation and provide maximal protection from ischemia-reperfusion injury to the ileum.

The expert technical assistance of Tri Phan and the graphic assistance of Max Turk are gratefully acknowledged. This work was supported by National Institutes of Health Grants RO1-DK-50745 and P50-GM-35529 and Department of Defense Disaster Relief and Emergency Medical Services grant to B. C. Kone.

REFERENCES


