Therapeutics for Acute Lung Injury: Time to call in the DRs?

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Summary sentence: Discussion on the possible benefit of dopamine receptor (DR) agonists in the treatment of ALI/ARDS identified by Bone et al.

Lung inflammation is a prominent feature of both acute and chronic respiratory syndromes. Classically, in conditions like non-pulmonary sepsis, pneumonia or chronic obstructive lung disease (COPD), this inflammation has been associated with infection, but may also result from other non-infectious insults, such as trauma or injury. Both infectious and non-infectious insults can lead to Acute Lung Injury(ALI)/Acute Respiratory Distress Syndrome (ARDS), where regardless of the underlying stimuli, the net result of the inflammation initiated is damage to the alveolar-capillary membrane which can result in respiratory failure [1]. As the underlying mechanisms unifying these diverse insults are not well understood, the development of new treatments has also lagged behind. Even where we think we understand the initiating stimulus, for example in sepsis, targeting inflammatory pathways with pleiotropic anti-inflammatory drugs such as steroids or aspirin provide little benefit and ALI/ARDS outcomes remain poor. Moreover, even β2-agonists, which might be thought to provide some benefit due to their bronchodilator action, have not been demonstrated to provide any improvement in clinical outcomes [2]. Thus, there is a pressing need to identify new therapeutic targets to reduce ALI/ARDS morbidity and mortality.

The study published by Bone et al [3] in this issue of the *Journal*, adds to the growing body of evidence obtained from animal models for the targeting of dopamine receptors (DRs) for the treatment of ALI. This study investigated the effects of the D1 receptor agonist, fenoldopam, on peritoneal macrophages and alveolar epithelial cells derived from a murine model of lipopolysaccharide (LPS)-induced lung injury. Their Initial experiments characterized the responses of

murine macrophages and human monocytes to stimulation with fenoldopam and demonstrated an increase in threonine phosphorylation of the enzyme, adenosine monophosphate kinase (AMPK).

AMPK is a central sensor and regulator of cellular metabolic and bioenergetic demand and has a role in preserving catabolic metabolism in activated inflammatory cells, resulting in modulation of inflammation [4]. This enzyme is a heterotrimer activated by both AMP binding and phosphorylation of the Thr172 residue of the alpha-subunit. Bone et al [3] demonstrate that AMPK phosphorylation by fenoldopam was mediated by the phospholipase C (PLC) pathway in peritoneal macrophages using both a specific activator (*m*-3M3) and inhibitor (U73122) of this pathway. As the PLC pathway classically results in PKC activation, these results suggest a possible role for protein kinase C in maintaining AMPK phosphorylation, as has been previously shown in the heart [5] (Figure 1). Bone et al [3] further demonstrated that AMPK phosphorylation was inhibited by treatment of the peritoneal macrophages with LPS, but phosphorylation was maintained when cells were treated with fenoldopam prior to LPS exposure. This effect of the D1R agonist on AMPK also appears to be coupled to its anti-inflammatory action, as the release of the pro-inflammatory cytokines TNFα and MIP-2 into supernatants was decreased in LPS-treated macrophages pretreated with fenoldopam.

The effects of fenoldopam on AMPK activation was not limited to macrophages as the authors also demonstrated a similar increase in AMPK phosphorylation in primary murine type II alveolar epithelial cells treated with this

D1R agonist. Furthermore, in a whole animal model of LPS-induced ALI, pretreatment of C57BL/6 mice with fenoldopam inhibited the LPS-induced increase in lung wet-to-dry ratio, accumulation of bronchoalveolar lavage (BAL) neutrophils, and protein exudate into the BAL. However, the authors demonstrated that fenoldopam had no effect on AMPK phosphorylation in isolated blood neutrophils, suggesting that this drug may have no direct effect on the infiltrating neutrophils in this ALI model. The reduction in lung inflammatory infiltrate observed is more likely a result of the observed decrease in LPS-induced pro-inflammatory cytokine levels in BAL from D1R agonist-treated mice. Again, similarly to the experiments using peritoneal macrophages, this reduction in cytokine release appears to be coupled to the inhibition of the LPS-induced decrease in AMPK phosphorylation by fenoldopam pretreatment.

In order to deconvolute the relative contributions of the lung epithelium and macrophages to these D1R controlled pathways, the authors conducted an elegant experiment that showed distinct cross talk between epithelial cells and macrophages. Primary alveolar epithelial cells were pretreated with fenoldopam for 4 h before being stimulated with IL-1β for a further 4 h. The cells were then washed, the media was replaced and conditioned media was harvested after a further 24 h. There was no TNFα released by the epithelial cells, but this cytokine was released by macrophages when this conditioned media was added. In line with the previous data presented in this paper, fenoldopam and another known AMPK activator, metformin, both prevented TNFα release by the macrophages. As lung macrophages are known producers of IL-1β in response

to LPS stimulation [6], these results suggest a positive feedback loop initiated by this paracrine crosstalk (Figure 1).

Thus the reduction in LPS-induced cytokine release in both macrophages and lung tissue is likely to be directly linked to the preservation of AMPK phosphorylation initiated by fenoldopam. Bone et al, demonstrated that phosphorylation of the downstream target of AMPK, acetyl-CoA carboxylase, was decreased in the lungs of LPS-treated animals but was preserved by fenoldopam pretreatment. LPS-treatment also decreased the expression of subunits of the mitochondrial electron-transport chain complexes I and V and again fenoldopam prevented this decrease. Taken together, these results suggest that by inducing activation of AMPK, fenoldopam preserves mitochondrial function in the lung, suggesting that the reduction in LPS-induced inflammation may be mediated by both direct (e.g. effects on NF-κB) and indirect (e.g. mitochondrial protection) effects on macrophages (Figure 1).

This study is not without its limitations. Firstly, only murine peritoneal macrophages and human monocytes were investigated in this study not lung macrophages, which may be important given the cell type specific effects noted by the authors. In addition, all of the fenoldopam effects reported are due to pretreatment of both cells and animals and thus may not necessarily translate into possible therapeutic applications. Moreover, despite this fenoldopam pretreatment in the *in vivo* LPS-challenge model, lung inflammation was only attenuated and not prevented.

Whilst this study adds to the emerging literature regarding the possible effects of both D1 and D2 receptor agonists in the lung [7-10], treatment with dopamine itself is not currently recommended by the severe sepsis guidelines [11]. Importantly, the inability of animal models to fully replicate the human syndrome of ALI/ARDS has already led to failures of promising targets identified using animal models [12]. Thus, a rush to investigate the use of DR agonists is probably premature. However, it may be possible to test whether the downstream target of these receptors, AMPK, is involved in ALI/ARDS as AMPK is also activated by the drug metformin. Given the increasing use of metformin to treat type II diabetes, a retrospective study of the outcomes of patients admitted for ALI/ARDS stratified by metformin treatment may shed some light on whether AMPK is an appropriate drug target to ameliorate the human disease. Such a study could then be used to inform the design of a prospective trial of agents that activate AMPK for efficacy in ALI/ARDS.

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Competing interests: KJS has no relevant conflicts of interest to declare.

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Figure Legend

Figure 1: D1R-mediated cross-talk between alveolar macrophages and epithelial cells. A) Stimulation of macrophages by bacteria or LPS causes activation of pro-inflammatory pathways that inhibit (red line) phosphorylation and thus activation of AMPK. At the same time, IL-1β is released by the macrophages which can act on nearby type II alveolar cells to release paracrine factors that can feedback onto the macrophages causing further release of TNF. **B)** In the presence of fenoldopam, AMPK phosphorylation and activation is preserved, possibly via phospholipase C(PLC)-mediated activation of protein kinase C (PKC), which reduces the LPS-induced inflammatory cascade in both macrophages and epithelial cells and preserves mitochondrial function in the macrophages.

Figure 1A

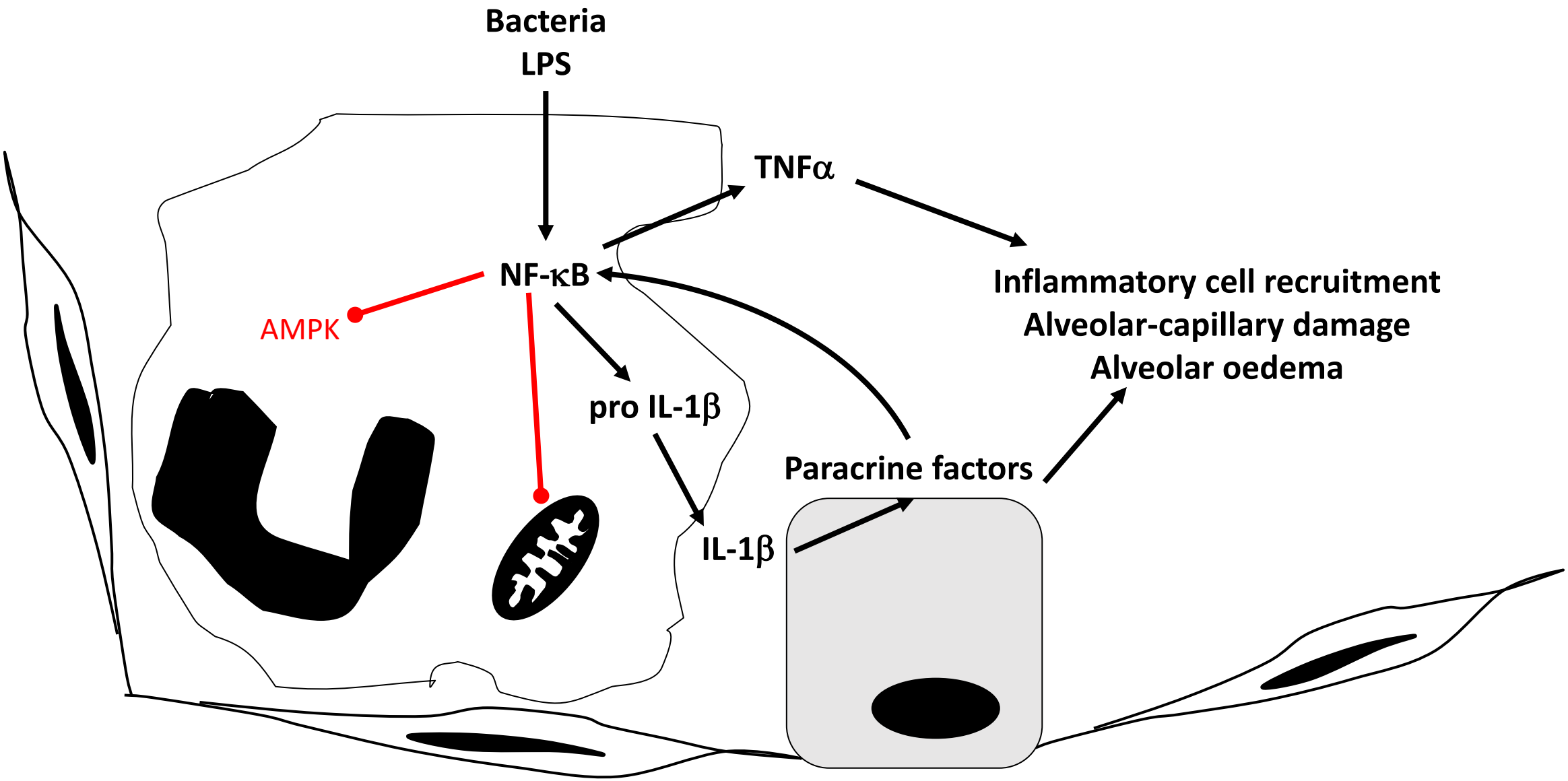
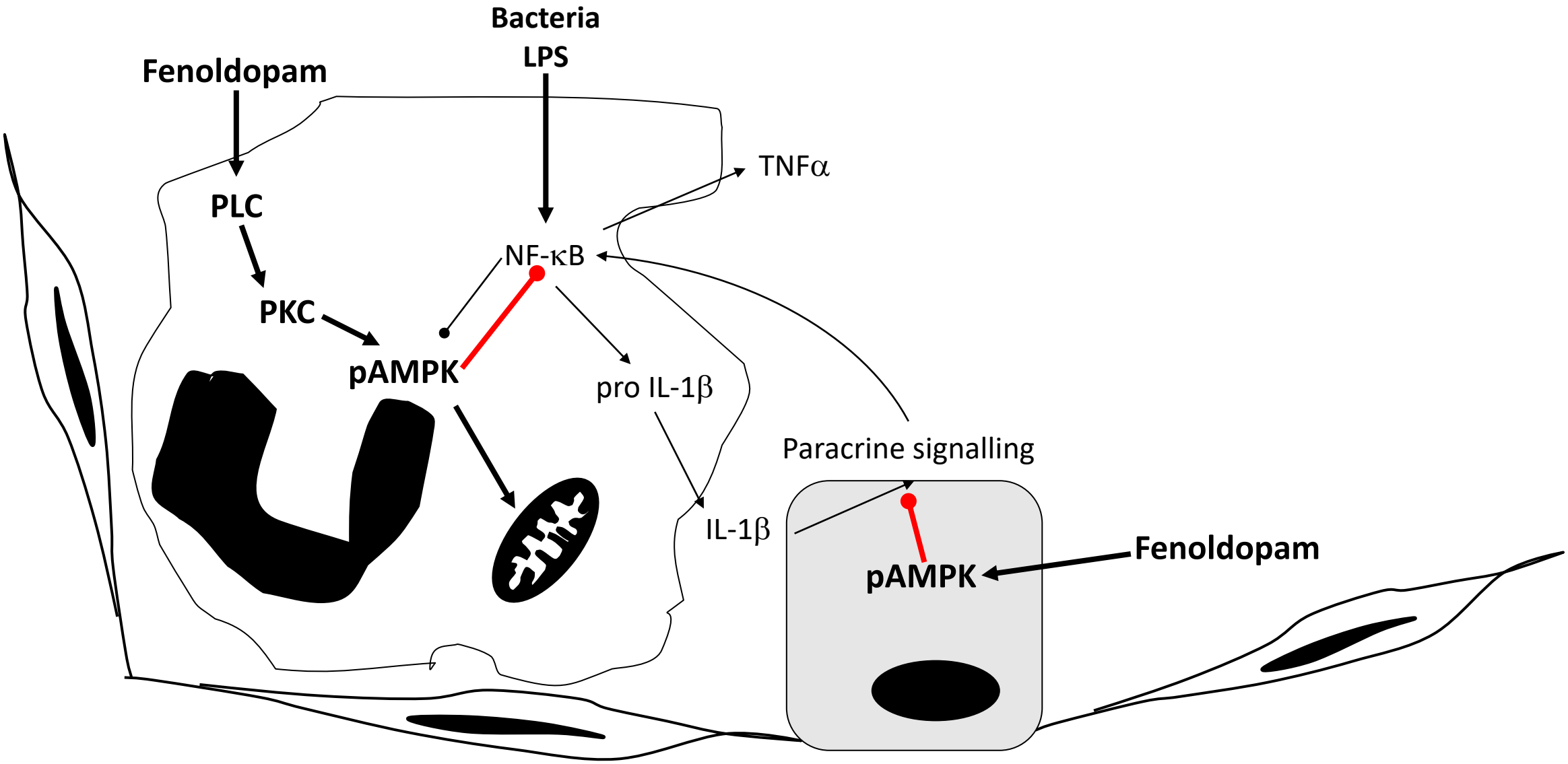

Bacteria
LPS
TNFα
NF-κB
AMPK
Inflammatory cell recruitment
Alveolar-capillary damage
Alveolar oedema
pro IL-1β
Paracrine factors
IL-1β

Figure 1B


Bacteria
LPS
Fenoldopam
TNFα
PLC
NF-κB
PKC
pAMPK
pro IL-1β
Paracrine signalling
IL-1β
pAMPK
Fenoldopam