PROKINETICINS PARTICIPATE IN DEVELOPMENT OF INFLAMMATORY PAIN

Giannini Elisa, Lattanzi Roberta, Campese Antonello*, Negri Lucia, Melchiorri Pietro

Department of Human Physiology and Pharmacology, *Department of Experimental Medicine and Phatology, University of Rome "La Sapienza", Italy

Bv8, prokineticin-1 (PK1) or EG-VEGF (endocrine gland-derived vascular endothelial growth factor), and prokineticin-2 (PK2), are naturally occurring peptide agonists of two G-protein-coupled receptors, prokineticin receptor 1 and 2 (PKR1 and PKR2). We showed that PK2 is highly expressed by neutrophils in two isoforms (PK2 and PK2L) and Bv8, its amphibian analogue, produces sensitization to thermal and mechanical stimuli acting on PKRs expressed in the dorsal root ganglions (1). Our hypothesis is that PK2 released by neutrophils recruited in inflamed tissues binds the PKRs on the primary sensitive neurons and lowers the nociceptive threshold contributing to inflammatory pain. We induced inflammation in one hind paw of rats by injecting complete Freund's adjuvant (CFA) and, at different time points (from 15 min to 30 days), we evaluated the development of paw oedema (plethysmometer, Ugo Basile), hyperalgesia (Randall Selitto, Ugo Basile) and we collected paw skin samples to perform quantitative real time RT-PCR (iCicler, BioRad). Our data demonstrate that inflammation induces a strong increase in both splice variants of PK2, with peak values reached from 9 and 24 h (PK2 1000 ± 90 fold over baseline; PK2L 700 ± 65 fold over baseline). The development and duration of hyperalgesia and of paw oedema (200% over baseline at 24h) correlated with the dramatic increase in PK2 expression. PKR1 expression showed a similar time-course (60 ± 6.5 fold over baseline at 24 h). PKR2 started to increase after 3 days and was still at maximum after 30 days.

To identify the cellular source of PK2, we performed ^{35}S-riboprobe labelled in situ hybridization on CFA inflammed paw sections. Strong signal (NTB nuclear emulsion Kodak) was seen in inflammed paws collected from 6 to 24 h, clearly associated with infiltrating/extravasated cells, possibly neuthophils and/or macrophages. Hence, we enzimatically dissected CFA inflammed paws (24h) to obtain purified populations of neutrophils and macrophages by FACS cell sorting; the quantity of infiltrating neutrophils was 5 times higher than that of macrophages. Real time RT-PCR analysis, performed using equal amount of RNA, showed that PK2 mRNA expression was 1200 ± 130 fold higher in neutrophils than macrophages. Taken together, our results demonstrate that prokineticins released within inflammed tissue may sensitize nociceptors by acting on PKRs.

1) Negri L., Lattanzi R., Giannini E., Metere A., Colucci M., Barra D., Kreil G., Melchiorri P. (2002). Br. J. Pharmacol., 137, 1147-1154.