AN ANTINOCICEPTIVE - OPIOID SYSTEM IN RAT ACHILLES TENDON

+*Ackermann, P W; +*Speta, M; +*Ahmed, M (A-Swedish Medical Research Council); +*Kreicbergs, A (A-Swedish Medical Research Council)
+*Karolinska Institutet, Stockholm, Sweden. Orthopedic Laboratory, Resarch Center M3:02, Karolinska Hospital, SE-171 76 Stockholm, SWEDEN, 46-8-51772699, Fax: 46-8-51776103, Paul.Ackermann@ort.ks.se

INTRODUCTION

Pain is one of the main indications of orthopedic surgery. Yet, very little is known about the mechanisms of pain originating from the locomotor system. This also applies to chronic pain of the Achilles tendon. Until now, substantial knowledge has accumulated on pain pathways in the central nervous system, whereas peripheral pain mechanisms have been less investigated [1]. The objectives of the present study on the Achilles tendon were (a) to investigate the neuronal occurrence of endogenous opioids, i.e. enkephalins, by immunohistochemistry; (b) to quantify enkephalins by radioimmunoassay (RIA) and (c) to identify and characterize opioid receptors by in vitro receptor binding assay (RBA).

MATERIALS AND METHODS

Experimental animals: Male Sprague-Dawley rats were used in the experiments, which were approved by the local animal ethics committee. The Achilles tendon was dissected bilaterally under anaesthesia and for immunohistochemistry they were fixed in 4% paraformaldehyde solution containing 0.2% picric acid while for RIA and RBA they were frozen.

Immunohistochemistry: Fixed tissues were sectioned (15 µm) and stained with primary antisera for Met-enkephalin (ME, 1:20,000), Leu-enkephalin (LE, 1:20,000), Met-enkephalin-Arg-Pro (MEAP, 1:20,000), Met-enkephalin-Arg-Gly-Lys (MEAGL, 1:20,000). Following incubation with the primary antisera, the sections were incubated with biotinylated goat-anti-rabbit antibodies. Finally, fluorescein isothiocyanate (FITC)-conjugated avidin was used for visualization of the immunoreaction.

Radioimmunoassay: Frozen tissues were boiled for 10 min in 2 M acetic acid. After homogenization, the samples were sonicated and centrifuged at 3,000xg for 15 min. The supernatant was lyophilized and redissolved in phosphate buffer [2]. Analysis of the tissue extracts focused on MEAP. The tracer for 15 min. The supernatant was lyophilized and redissolved in phosphate buffer [2]. Analysis of the tissue extracts focused on MEAP. The tracer used for RBA. Aliquots of membrane homogenates (0.5-0.7 mg/ml protein) were incubated with $[^3H]$naloxone for 30 min at 25°C. The reaction was terminated by rapid filtration under vacuum through GF/B Whatmann filters pretreated with 0.1% polyethyleneimine (PEI). The bound radioactivity was measured using a $[^3H]$-counter. Non-specific binding was measured in the presence of 10 μM unlabeled naloxone. The protein concentration was determined by the method of Lowry [3]. Scatchard analysis was performed by first-order non-linear regression analysis of the data from saturation isotherms. The binding parameters dissociation constant ($K_d$) and receptor density ($B_{max}$) were calculated from these plots. The given values represent the means ± SEM.

RESULTS

Identification and quantification of enkephalins: Nerve fibers immunoreactive to all tested enkephalins, i.e. ME, LE, MEAP and MEAGL, were identified in the Achilles tendons. They predominantly occurred in the paratenon and the connective tissue, where they were mostly found to surround the blood vessel walls (Fig. 1). Among the identified enkephalins, quantification by radioimmunoassay was only done for MEAP. The concentration was 0.108 ± 0.082 fmol/mg wet tissue.

Characterization of opioid receptors: The tissue possessed opioid binding sites, shown with the non-selective opioid ligand $[^3H]$naloxone. The binding of $[^3H]$naloxone was specific and saturable (Fig. 2). The equilibrium dissociation constant ($K_d$) for the identified binding sites disclosed high affinity ($K_d$: 5.52 ± 0.89 nM), but low binding capacity ($B_{max}$: 5.95 ± 0.96 fmol/mg protein). The Hill coefficient ($n_H$) value was 1.01 ± 0.21, indicating a non-cooperative nature of the binding process and also that $[^3H]$naloxone can interact with multiple classes of binding sites with equal affinity.

REFERENCES