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BINDING OF [^{3}H]DYNORPHIN A TO APPARENT κ OPIOID RECEPTORS IN DEEP LAYERS OF GUINEA PIG CEREBRAL CORTEX

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Dynorphin A and its fragments (e.g. dynorphin A-(1-13)) potently displace prototypical alkaloid ligands for the κ subtype of opioid receptor in rat (Quirion and Pert, 1981) and guinea pig (Chavkin et al., 1982; Corbett et al., 1982) brain membranes. However, we and others (Quirion and Pert, 1981; Corbett et al., 1982) have found that dynorphin A-related peptides also exhibit substantial potency in displacing prototypical μ and δ receptor ligands from brain membranes. Since these ligands are not completely selective, it is difficult to distinguish whether dynorphin acts at the μ and δ sites or if the radioligands bind to the κ receptor. This issue has been clarified for guinea pig brain: [^{3}H]dynorphin A binds specifically to guinea pig brain membranes and appears to selectively label κ sites (Young et al., 1983). These sites, when labelled by [^{3}H]ethylketocyclazocine or [^{3}H]bremazocine, are concentrated in the deep layers of guinea pig cerebral cortex (Goodman and Snyder, 1982). Since these tritiated alkaloids exhibit little receptor selectivity, excess concentrations of μ and δ ligands are included in the incubation medium to ensure that only κ sites are available for labelling (Corbett et al., 1982; Goodman and Snyder, 1982). However, the concentration of morphine (30 nM) used by Goodman and Snyder (1982) may have been insufficient to saturate the μ sites (cf. Corbett et al., 1982). Since [^{3}H]dynorphin A exhibits high κ selectivity in guinea pig brain (Young et al., 1983), we carried out the present study to determine whether specific binding sites for this ligand are distributed in the deep cortical layers.

Adult male Hartley guinea pigs were deeply anesthetized with pentobarbital and their brains were rapidly removed and frozen. Twenty-μm coronal sections of the brains were cut in a Bright cryostat at −16°C and thaw-mounted onto gelatin-coated microscope slides. The sections were dried at 0°C under vacuum and stored at −70°C before being warmed to 4°C for assay. The sections were covered with 200 μl of 0.05 M Tris · HCl (pH 7.55) containing 2.5 nM [^{3}H]dynorphin A for 90 min at 4°C (Young et al., 1983). Nonspecific binding was evaluated by incubating adjacent sections in the same medium also containing 10 μM UM-1071 (the active stereoisomer of MR 2034, a potent κ agonist; see Young et al., 1983). The slides were then washed by continuous agitation in 4 changes (2 min each) of 200 ml of 0.05 M Tris HCl (pH 7.55) at 4°C, rapidly dried under a stream of cool air, and exposed in an X-ray cassette to tritium-sensitive LKB Ultrofilm for 10 weeks. The film was then developed for autoradiography according to the manufacturer's directions, and photographed using an enlarger.

[^{3}H]Dynorphin A binding to guinea pig brain sections, as determined by liquid scintillation counting of the sections after agitation for 1 h in scintillant, was displaced approximately 45 percent by 10 μM UM-1071, a κ ligand previously shown to specifically (and selectively) displace [^{3}H]dynorphin A binding in guinea pig brain membranes (Young et al., 1983). Autoradiographs of the sections revealed a concentration of [^{3}H]dynorphin A binding sites in the deeper layers (predominantly V and VI) of cerebral cortex (fig. 1A), although there were some regional variations in binding site

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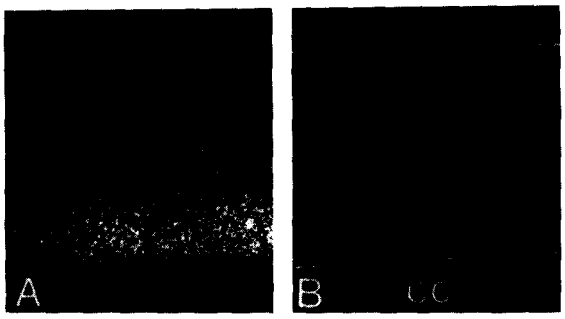

Fig. 1. Distribution of [^{3}H]dynorphin A binding sites in a coronal section of guinea pig cerebral cortex (parietal region). Autoradiographs show binding in the absence (A) and presence (B) of 10 μM UM-1071, a potent κ agonist. The concentration of [^{3}H]dynorphin A binding sites in the deeper layers is blocked by UM-1071, while the residual nonspecific binding is uniformly distributed throughout the cortical laminae.

density. The concentration of sites in the deeper cortical layers was not shown in sections exposed to 10 μM UM-1071 (fig. 1B), demonstrating the specificity of this regional distribution of binding sites.

The localization of specific [^{3}H]dynorphin A binding sites in the deep layers of guinea pig cerebral cortex is consistent with previous reports that alkaloid-labelled κ opioid receptors are similarly distributed (Goodman and Snyder, 1982) and that dynorphin A appears to be κ-selective in guinea pig brain tissue (Chavkin et al., 1982; Corbett et al., 1983; Young et al., 1983). μ and δ sites in contrast, appear to have a different localization within guinea pig cerebral cortex (Goodman and Snyder, 1982). Parenthetically, we have found it difficult to obtain UM-1071-displaceable [^{3}H]dynorphin A binding in rat brain sections, which may be consistent with the reported lack of κ selectivity of dynorphin in rat brain (Quirion and Pert, 1981).

The pharmacological significance of κ receptors in the deep cortical layers of guinea pig brain is unknown. These receptors have been suggested to mediate the sedative effects of κ alkaloids (Goodman and Snyder, 1982), an idea which requires experimental testing. The significance of cerebral cortical κ opioid receptors may become clearer when their distribution is studied in relation to endogenous opioid neural pathways.

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