Somatostatin, Substance P and [Lys]-Vasopressin Interfere with the Binding of [3H][D-Ala2, Met5]Enkephalinamide to Rat Brain Membranes

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Abstract—Somatostatin and substance P (10^-6–3x10^-5 M) inhibited dose-dependently the specific binding of [3H][D-Ala2, Met5]enkephalinamide ([3H]-DALAMID) to rat brain membranes, by decreasing the number of binding sites. [Lys]-Vasopressin also inhibited the binding, by affecting both the number of binding sites and the affinity of receptors to [3H]-DALAMID. The results indicate that somatostatin and substance P may interfere with the endorphin system by an apparently competitive manner.

The neuropeptides constitute the newest class of molecules considered to play key roles as transmitters or modulators in the central and peripheral nervous system. The list of these peptides is readily growing and now includes more than 30 different molecules, some of them being structurally related. Terenius (1) reported that adrenocorticotropic hormone (ACTH) and somatostatin were peptides with partial antagonist-like selectivity to the endorphin system. In this communication, possible neuropeptides interacting with the opioid receptors in rat brain membranes were investigated through the inhibition of [3H]-DALAMID binding.

The following peptides were used: angiotensin II (human), bombesin, bradykinin, cholecystokinin, [D-Ala2, Met5]-enkephalinamide (DALAMID), neurotensin, somatostatin, substance P, vasoactive intestinal polypeptide (porcine), [Lys]-vasopressin, which were purchased from Peptide Institute, Inc., and [Arg]-vasopressin from Sigma Chemicals. All peptides were used without further testing for purity.

Membrane fractions (2) from rat brain (0.3–0.4 mg protein) were incubated in 1 ml of 50 mM Tris-HCl buffer (pH 7.5) containing 0.1 mM MnCl2 (3 and M. Maruyama, unpublished data), 0.1 mM phenylmethylsulphonylfluoride (PMSF), 1 mM NaN3, 20 

Somatostatin, substance P and [Lys]-vasopressin (10^-6–3x10^-5 M) inhibited, dose-dependently, the specific binding of [3H]-DALAMID (2x10^-9 M) (Fig. 1A). [Arg]-vasopressin (3x10^-5 M) inhibited the binding about 10% as much as [Lys]-vasopressin. Other peptides examined had no effect on the specific binding of [3H]-DALAMID. NaCl (100 mM) had no effect on the inhibition by these three non-opioid peptides. The inhibition by these three non-opioid peptides (3x10^-5 M) was at most 50–60% of the specific [3H]-DALAMID binding, whereas the IC50 (the concentration producing 50% inhibition) of unlabelled DALAMID was 2x10^-9 M. The high concentration of these peptides required for the inhibition of [3H]-DALAMID binding was not due to the reduction of the apparent concentration of

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these peptides by the inactivation of these peptides. Negligible degradations of non-opioid peptides occurred during the binding assay, which was ascertained by HPLC analysis (data not shown). Figure 1B shows the Scatchard plots of the specific [3H]-DALAMID binding in the presence of these three peptides. The binding inhibition by somatostatin (10^-5 M) or substance P (3x10^-5 M) was mainly due to the decrease in the number of binding sites of [3H]-DALAMID; thus, it seems to be apparently competitive. The decrease in the number of binding sites was observed to be in a dose-dependent manner (data not shown). On the other hand, the effect of [Lys]-vasopressin (10^-5 M) was due to both the decrease in the number of binding sites and the change in the affinity of receptors to [3H]-DALAMID. Thus, it seems to be non-competitive.

Until now, we have had no direct evidence for elucidating the physiological and/or pharmacological meanings of the present results. Particular interest has been focused on the interaction between opioid peptides and substance P on the basis of a considerable overlap of nerve terminals containing enkephalins and substance P, particularly in the area related to pain and analgesia (See, for example, the review of Pernow (4)). Mayer et al. (5) reported that [D-Ala^2, Met^5]enkephalin enhanced the binding of [125I][Tyr^8]-substance P to synaptic vesicles of rat brains in a cooperative way. Terenius (1) has reported that somatostatin (10^-6-3x10^-5 M) inhibited the binding of [3H]dihydromorphine and [3H]-naloxone to rat brain membranes and postulated that it may function as an endogenous antagonist to the endorphin system. Berson et al. (6) reported that a systemically administered high concentration of [Lys]-vasopressin exerted a potent, dose-dependent and naloxone-resistant antinociceptive effect. It is well known that the clustering of receptors is observable by the binding of the peptidergic ligands to their receptors (7). The possible cluster formation by non-opioid peptides may interfere with the [3H]-DALAMID binding to opioid receptors. The change of membrane fluidity modulated the binding characteristics of opioid ligands to their receptors in rat brain membranes (M. Maruyama, unpublished data). Thus, although the concen-
tration of non-opioid peptides required to inhibit the [\(^3\)H]DALAMID binding seems to be considerably higher as compared to that of unlabelled DALAMID (IC\(50 = 2 \times 10^{-9}\) M) and the mechanism of the inhibition remains still unknown. Such interaction between non-opioid and opioid peptides as shown in the present study deserves some attention from the pharmacological point of view.

References