MOLECULAR BASIS FOR DYNORPHIN A SELECTIVITY: A CHIMERIC STUDY

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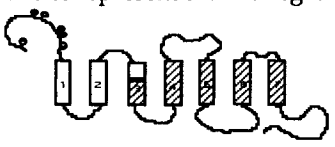
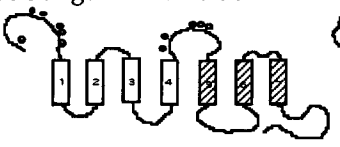
The role of Coulombic interactions (1,2,3,4) in the selectivity of DynA fragments toward the κ receptor was investigated by the construction of several κ/δ receptor chimeras. The affinity of different Dyn fragments to these chimeric as well as wild type receptors were determined by competition study. Based on the binding profile of DynA fragments at different receptors, it is suggested that while the δ receptor may contain a high affinity pocket for the Tyr-Gly-Gly-Phe opioid core, the Coulombic interactions between the positive charges in the Dyn peptides and the negative charges in the extracellular (EC) domains of the κ receptor may play an important role in the selectivity of DynA toward the κ receptor.

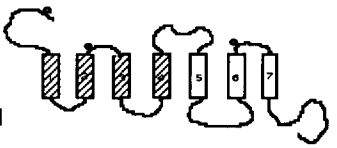
Restriction sites Afl3 and Bgl2 present in both the rat κ and δ receptor cDNA (4, 5) were utilized to produce chimeric κ/δ receptors as illustrated in the following figure. Small circles represent the net negative charges in the EC domains.



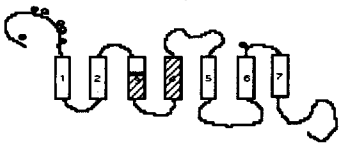
A: κ 1-141, δ 132-372



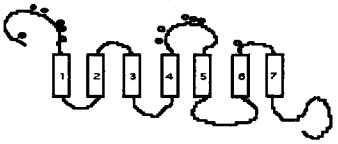
B: κ 1-227, δ 215-372



C: δ 1-214, κ 228-380



D: κ 1-141, δ 132-214, κ 228-380



Wild type κ (1-380)

Wild type δ (1-372)

The cDNA of these receptors were subcloned into a pCMV-neo expression vector and plasmid DNA was transfected into COS-1 cells using the method of Chen and Okayama(6). Receptor binding was performed according to Goldstein and Naidu (7) but the binding of the peptides was carried out at 0°C to minimize degradation. About 1.5 nM of [^{3}H]EKC (24.8 Ci/mmol, NEN) was used to label the receptors. The results of competition studies are summarized in the following table (apparent Ki, nM).

	Sequence	A	B	C	D	κ	δ
DynA(1-5)	YGGFL	5.0	15	5200	15,000	2800	0.8
DynA(1-6)	YGGFLR	8.4	5.5	360	3300	60	1.9
DynA(1-7)	YGGFLRR	5.8	0.70	130	360	0.7	1.1
DynA(1-8)	YGGFLRRI	10	0.75	111	260	0.54	2.9
DynA(1-9)	YGGFLRRIR	12	0.27	90	100	0.20	1.4
DynA(1-11)	YGGFLRRIRPK	5.0	0.10	207	150	0.15	1.4

All of the receptors showed good binding to the non-selective alkaloid EKC, but their affinity toward the DynA fragments varied greatly. The δ receptor showed good affinity and

chimeric receptor A showed moderate affinity for all DynA fragments tested, irrespective of charge --the C-terminal tail of the DynA peptides seem to have little influence on their affinity toward these two receptors. By contrast, the affinity of the DynA fragments for the κ receptor and chimeras B, C, D is highly dependent on the C-terminal tail of the peptides. A general tendency is that the affinity becomes higher as the number of positive charges increases in the C-terminal tail of the DynA fragments. From Leu-ENK to DynA (1-11), the affinity increased about 20,000-fold for the κ receptor, while the affinity increase is around 100-fold for the other three chimeras. The addition of the first two arginine residues to Leu-ENK seems to have the greatest effect on binding affinity.

The κ receptor contains a total of twelve net negative charges in its extracellular domains, while the δ receptor only has two net negative charges in its EC domains (4). The simplest way to explain the selectivity of the proDyn peptides to the κ receptor is to propose the interaction between the positive charges in their C-terminus and the negative charges in the EC domains of the κ receptor play an important role in binding. If this hypothesis is correct, one would expect that, for chimeras containing the regions that interact with the positive charges, an increase in the affinity of DynA fragments should occur as the positive charges are added into the C-terminal tails of the peptides. In addition, one also needs to explain the high affinity of DynA fragments toward the δ and the μ receptors. Our results are consistent with the Coulombic interaction hypothesis. It seems that regions containing EC loops 2 and 3 of the κ receptor may be more critical than the N-terminal extracellular domain in the interaction with the positive charges of the DynA fragments. The combined effects of these two domains may largely account for the affinity gained by the wild type κ receptor. We can also conclude that there is a high affinity Tyr-Gly-Gly-Phe binding pocket in the δ receptor, This pocket is probably mainly located in the TM5-TM7 region of the δ receptor. In contrast, the corresponding binding pocket may be relatively weak in the κ receptor, with the binding of DynA being more dependent on the extracellular domains than on the Tyr-Gly-Gly-Phe binding pocket. This also helps to explain why the short DynA fragments, as they lose their positive charges, are no longer selective across opioid receptors. While we have focused in this discussion on the potential charge interactions, we would like to emphasize that other important interactions are likely to exist between the extracellular loops of κ and the proDyn peptides. Specific mutations should be useful in pinpointing the role played by single amino acids or restricted regions of the κ extracellular domains in ligand selectivity.

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