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Rapid communication

INTERACTION OF DES-TYROSINE-γ-ENDORPHIN (DTγE, β-LPH$_{62-77}$) WITH NEUROLEPTIC BINDING SITES IN VARIOUS AREAS OF RAT BRAIN

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Des-tyrosine-γ-endorphin (DTγE, β-LPH$_{62-77}$) induces in experimental animals effects which are comparable in some respects to those of neuroleptic drugs (De Wied et al., 1978). The peptide, however, appeared to be devoid of the typical locomotor and sedative effects of these drugs. On the basis of these observations it was postulated that DTγE might possess antipsychotic activity in humans. Indeed, the first clinical studies on schizophrenic patients at least partly resistant to neuroleptic medication showed that particularly the hallucinations and delusions diminished or even disappeared within a few days of daily DTγE treatment (Van Ree et al., 1978). It was thus of interest to investigate the possible interaction of DTγE and of related LPH-fragments with the in vitro binding of various neuroleptics and other centrally acting drugs to their respective binding sites in rat brain.

The procedures of the neuroleptic binding assays have been described in detail (Leysen et al., 1978a,b). LSD, fentanyl, naloxone, muscimol, dexetimide and diazepam binding assays were performed using a technique similar to that used for the neuroleptic drugs. In brief, a microsomal fraction was prepared from fresh rat brain regions and incubated in triplicate in a volume of 0.55—2.2 ml buffer (0.1—0.6 mg protein/ml) in the presence of 2—4 nM of ^{3}H-ligand and various concentrations of the peptides. Membrane-bound ^{3}H-ligand was separated using the rapid filtration technique and radioactivity was counted subsequently. Some details of the assays are presented in table 1. The following peptides were used: β-LPH$_{61-76}$ (α-endorphin), β-LPH$_{62-76}$ (DTαE), β-LPH$_{62-77}$ (DTγE), β-LPH$_{78-91}$, ACTH$_{4-10}$ (β-LPH$_{47-53}$) and ACTH$_{4-10}$ (D-Phe^7).

None of the six peptides tested were able to displace ^{3}H-haloperidol or ^{3}H-spiperone from their stereospecific binding sites in membrane preparations of rat striatum or frontal cortex respectively (table 1). The same holds for the ^{3}H-spiperone binding in the accumbens area in the presence of both R 43448 (labelling predominantly dopaminergic antagonist sites) and 5,6-dihydroxy-2N,N-dipropyl-aminotetraline (labelling mainly serotonergic antagonist sites) (Leysen, 1978b). The endorphin fragments did not inhibit ^{3}H-LSD and ^{3}H-diazepam binding. Fentanyl binding was inhibited by the opioid peptide α-endorphin in a dose-dependent manner (IC_{50}: 2×10^{-7} M), less effectively by β-LPH$_{78-91}$ and ACTH$_{4-10}$ (D-Phe^7) and was not inhibited by the other endorphin fragments. Similar results were obtained with naloxone binding. These findings are in agreement with previous data, showing that removal of the tyrosine from α- and γ-endorphin leads to a loss of opioid activity of these endorphins (De Wied et al., 1978).

TABLE 1

Inhibition of specific binding of different ^{3}H ligands to membrane preparations of various brain areas by endorphin and ACTH fragments. Results are expressed as percentage inhibition in the presence of the highest concentration of the peptide indicated.

Ligand (spec. radioactivity, Ci/mMol)	^{3}H-Haloperidol (11)	^{3}H-Spiperone (23,7)	^{3}H-Spiperone (23,7) (+10^{-7} M R 43448)	^{3}H-Spiperone (23,7) (+10^{-7} M 5,6-dihydroxy-2N,N-dipropyl aminotetraline)	^{3}H-LSD (16)	^{3}H-Fentanyl (9)	^{3}H-Naloxone (18.5)	^{3}H-Diazepam (39)
Brain area	Striatum	Frontal cortex	Accumbens area	Accumbens area	Cerebral cortex	Forebrain	Brain minus cerebellum[3]	Frontal cortex
Receptor type	DA antagonist	5-HT antagonist	DA antagonist	5-HT antagonist	5-HT antagonist/ agonist	Opiate agonist	Opiate antagonist	Diazepam
Total binding (pmol/mg protein)	1.23	1.17	1.24	1.20	0.90	0.34	0.17	1.49
% spec. binding[1]	70[a]	54[a]	29[a]	46[a]	88[b]	58[c]	78[d]	95[e]
Concentration range of peptides (M)	$10^{-9} - 10^{-5}$	$10^{-9} - 10^{-5}$	$10^{-9} - 10^{-5}$	$10^{-9} - 10^{-5}$	$10^{-7} - 10^{-5}$	$10^{-9} - 10^{-5}$	10^{-7}	10^{-5}
Incubation/ conditions	2.2 ml, 37° C, 10 min	2.2 ml, 37° C, 10 min	1.1 ml, 37° C, 10 min	1.1 ml, 37° C, 10 min	2 ml, 37° C, 10 min	2.2 ml, 37° C, 10 min	2 ml, 0° C, 30 min	0.55 ml, 0° C, 30 min
β-LPH_{61-76}	< 10	< 10	< 10	< 10	< 10	95	28	< 10
β-LPH_{62-76}	< 10	< 10	n.d.[2]	n.d.	< 10	< 20	< 10	< 10
β-LPH_{62-77}	< 10	< 10	< 10	< 10	< 10	< 20	< 10	< 10
β-LPH_{78-91}	< 10	< 10	n.d.	n.d.	n.d.	45	< 10	< 10
$ACTH_{4-10}$	< 10	< 10	< 10	< 10	n.d.	< 20	< 10	< 10
$ACTH_{4-10}$ (D-Phe^7)	< 10	< 10	< 10	< 10	n.d.	23	n.d.	n.d.

[1] In the presence of 10^{-6} M (+)-butaclamol (a), LSD (b), dextromoramide (c), naloxone (d), diazepam (e).
[2] n.d. = not determined.
[3] Cytoplasmic fraction.

The interaction of the ACTH-like peptides at high concentrations with opioid receptors has also been described previously. In addition there was no interaction of DTγE with ^{3}H-muscimol (labelling GABA agonist sites) and ^{3}H-dexetimide (labelling muscarine cholinergic sites) binding in membrane preparations of rat cerebellum or rat striatum respectively. Also in experiments performed at 0°C or in presence of bacitracin (100 μg/ml), the peptides did not markedly interfere with the ^{3}H-ligand binding to neuroleptic sites.

The present data indicate that none of the endorphin and ACTH fragments tested showed high affinity binding to the neuroleptic sites in a number of rat brain areas and therefore can probably not be regarded as endogenous ligands for these sites. In particular it is of interest that the fragment DTγE although beneficial in schizophrenic patients, does not interfere with the in vitro binding of neuroleptics to brain tissue. The degree of inhibition of binding by neuroleptics is suggested to be related to the pharmacological and clinical potency of these drugs (Leysen et al., 1978a). Thus, if the effects of DTγE on psychosis are mediated by dopaminergic or serotonergic transmission in certain brain areas, it is likely that the site of action of DTγE is different from that of the currently used neuroleptics.

Neuroleptics have been initially characterized by their action on psychomotor function in humans and their reduction of psychotic symptoms in patients (Delay and Deniker, 1961). Animal experiments showed that neuroleptics induced typical and specific effects such as catalepsy and blockade of dopamine-like activity. Neither its pharmacological characteristics nor the biochemical properties just described allow DTγE to be designated at present as a typical neuroleptic drug.

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