Neuropeptides 5: 161-164, 1984

COMPARISON OF "SELECTIVE" OPIATE RECEPTOR ANTAGONISTS ON THE ISOLATED MOUSE VAS DEFERENS

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ABSTRACT

The selectivity and relative potencies of opiate receptor antagonists were compared on the mouse vas deferens preparation. ICI-174864 was found to be a highly selective antagonist at delta opiate receptors equal in potency to naltrexone in blocking the actions of delta agonists. Although less potent than naltrexone, beta-funaltrexamine (beta-FNA) and Mr-1452, like naltrexone, were less selective in that they blocked the actions of mu, delta and kappa agonists. The relative potencies of beta-FNA and Mr-1452 in antagonizing the three types of agonists also were similar to naltrexone.

INTRODUCTION

The classification of opiate receptors is based in part upon the relative potencies of agonists as well as the selectivity of antagonists [e.g. 1,2]. In recent years, drugs have been sought which will block selectively various receptors such as mu, kappa or delta receptors; and the isolated, electrically stimulated mouse vas deferens preparation commonly has been used to evaluate these agents. Beta-FNA is a naltrexone derivative which is thought to be a selective antagonist of mu receptors [3,4]. Mr-1452 [(-)-5,9 alpha-dimethyl-2(3-methyl-3-furyl)-2'-hydroxy-alpha-5,9-dimethyl-6,7-benzomorphan] is a furyl benzomorphan derivative which was reported to be selective for kappa receptors [5]. ICI-174864 [N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH] is a pentapeptide which blocks delta receptors [6]. The purpose of the present study was to compare the antagonist actions of beta-FNA, ICI-174864, and Mr-1452 to those of naltrexone upon the mouse vas deferens preparation. Interactions with mu, kappa and delta receptor agonists were evaluated.

METHODS

Male, albino ICR mice, weighing betwen 25 and 30 g, were sacrificed by decapitation. The vasa deferentia were removed, and 1.5 cm segments were suspended in organ baths which contained 30 ml of a modified Krebs' physiological buffer. The buffer contained the following (mM): NaCl, 118; KCL, 4.75 ; $CaCl_2$, 2.54; $MgSO_4$, 1.19; KH_2PO_4, 1.19; glucose, 11 ; $NaHCO_3$, 25, pargyline HCl, 0.3; tyrosine, 0.2; ascorbic acid, 0.1; and disodium edetate, 0.03. The buffer was saturated with 95% O_2-5% CO_2 and kept at 37 °C. The segments were attached to strain gauge transducers and suspended between two platinum electrodes. After a 30-min equilibration period, the segments were

stimulated once every 10 sec with pairs of pulses of 2 msec duration, 1 msec apart and at supramaximal voltage. Antagonists were added 10 min before the determination of cumulative concentration-effect relationships for the various agonists. The following agonists were studied: DSLET [Tyr-D-Ser-Gly-Phe-Leu-Thr], DADLE [D-Ala^2-D-Leu^5 enkephalin], LY-123502 [Tyr-D-Ala-Gly-4-F-Phe-phenyl-glycinamide acetate], morphine sulfate, FK-33824 [Tyr-D-Ala-Gly-MePhe-Met-(O)-ol], and Mr-2033 [(+)-5,9-beta-dimethyl-2'-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphan]. EC 50's were calculated by probit analysis, and pA_2 values were determined to assess relative potencies of antagonists [7]. All values are the means of n determinations ± the standard error of the mean.

RESULTS

ICI-174864 was a highly selective antagonist of delta receptors in the mouse vas deferens preparation. Low concentrations of this drug caused parallel shifts to the right in the concentration-effect curves for DSLET, DADLE and LY-123503 (Fig. 1). In contrast, in concentrations up to 3×10^{-6} M it did not block the actions of morphine, FK-33824 or Mr-2033. The pA_2 values obtained with DSLET (7.90±0.06), DADLE (7.60±0.03) and LY-123502 (7.70±0.51) did not differ appreciably which suggests that ICI-174864 blocks a single type of opiate receptor. Similar pA_2 values were obtained for naltrexone against DSLET (7.60±0.10), DADLE (7.65±0.05) and LY-123502 (7.57 ± 0.05). In contrast to ICI-174864, naltrexone was a potent antagonist of morphine (pA_2=8.59±0.15), Mr-2033 (pA_2=8.20±0.35) and FK-33824 (pA_2=8.00±0.35). In these experiments the slopes of the Schild plots did not deviate significantly from unity.

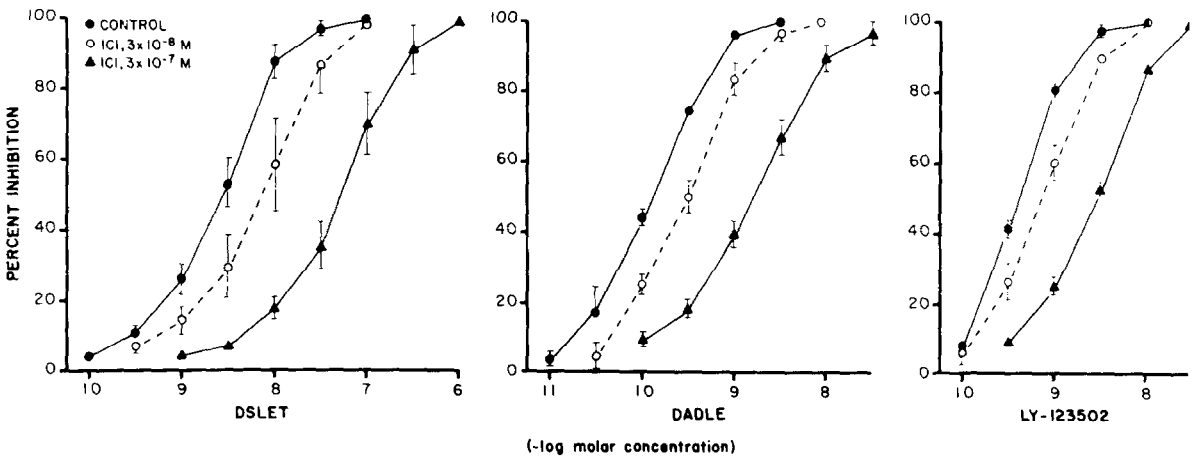

Fig. 1. Antagonism by ICI-174864 of the effects of DSLET, DADLE and LY-123502 upon the isolated, electrically stimulated mouse vas deferens preparation. Ordinate: inhibition expressed as a percent of the baseline contraction; abscissa: concentration of agonist. Solid circles: control preparations. Concentrations of antagonist are shown on each graph. Each point represents the mean of at least 3 determinations. Vertical lines represent standard errors of the mean.

Beta-FNA and Mr-1452 resembled naltrexone in that they blocked the inhibition of the contraction of the vas deferens produced by mu, kappa, and delta agonists. Beta-FNA shifted the morphine concentration-effect curve to the right and reduced the maximum responses to morphine (Fig. 2). It produced parallel shifts to the right in the concentration-effect curves for Mr-2033

and DSLET. pA_2 values for beta-FNA differed for DSLET, Mr-2033 and morphine which suggests that beta-FNA acts at multiple receptor sites (Table 1). Similar results were obtained with Mr-1452. Both beta-FNA and Mr-1452 were less potent than naltrexone in antagonizing each of the three agonists although the relative potencies of the three antagonists were similar.

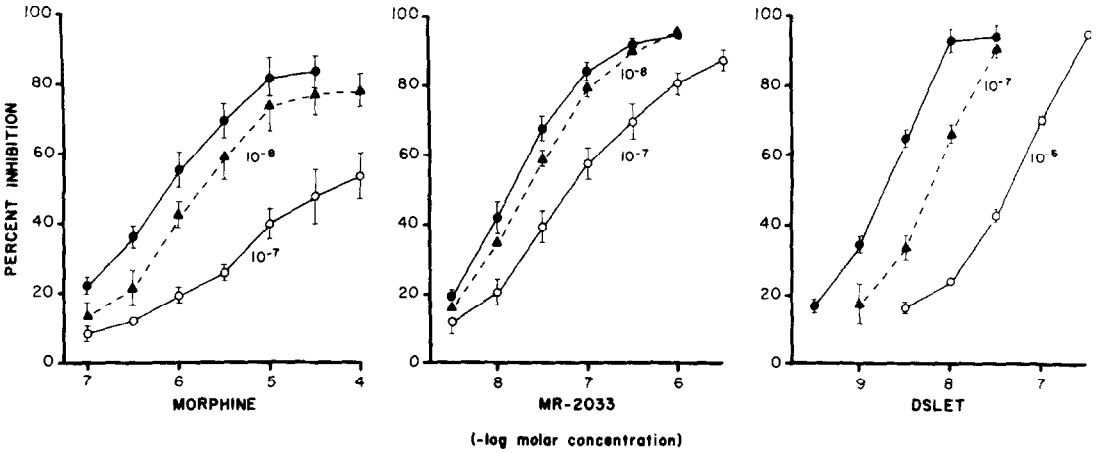

Fig. 2. Antagonism by beta-FNA of the effects of morphine, Mr-2033 and DSLET upon the isolated, electrically stimulated mouse vas deferens preparation. See Fig. 1 for details.

Table 1. pA_2 values of various opiate antagonists determined for mu, delta and kappa receptor agonists.

	Agonist					
	Morphine		Mr-2033		DSLET	
Antagonist	pA_2	slope	pA_2	slope	pA_2	slope
Naltrexone	8.60 ±0.15	1.12 ±0.21	8.11 ±0.24	1.14 ±0.17	7.60 ±1.10	1.18 ±0.14
Mr-1452	8.34 ±0.05	0.86 ±0.08	7.65 ±0.04	1.48 ±0.06	7.08 ±0.01	0.91 ±0.01
Beta-FNA	7.71 ±0.03	1.10 ±0.04	7.41 ±0.04	1.50 ±0.06	7.23 ±0.17	1.41 ±0.24
ICI-174864	< 5.52		< 5.52		7.90 ±0.06	1.01 ±0.08

Each value represents determinations on a minimum of 3 pairs of tissues at each of 3 concentrations of antagonist. Shown are mean values ± S.E.M.

DISCUSSION

ICI-174864 is a highly selective antagonist of the delta opiate receptor on noradrenergic neurons in the mouse vas deferens. Previously this compound was reported to be as potent as naloxone in antagonizing the actions of [D-Thr^2-Leu^5-Thr^6]-enkephalin (DTLET) and [Leu^5]-enkephalin, delta agonists, but to be inactive in concentrations up to 5×10^{-6} M against normorphine, a mu agonist, and tifluadom, a kappa agonist. The present study shows that ICI-174864 is as potent as naltrexone in antagonizing the delta receptor agonists, DSLET, DADLE and LY-123502, but is inactive against the mu agonists, morphine

and FK-33824, and the kappa agonist, Mr-2033.

In contrast, beta-FNA blocks the actions of agonists on mu, kappa and delta receptors in the vas deferens, although it is somewhat less potent than naltrexone. The differences in the pA_2 values for beta-FNA determined with drugs selective for the various receptor types supports the conclusion that beta-FNA blocks multiple types of opiate receptor. Previously beta-FNA has been reported as a highly selective antagonist of the mu receptor in the vas deferens [4]. In those experiments, tissues were exposed to beta-FNA for longer periods of time, and the antagonist was washed from the organ baths prior to testing for antagonism. It is possible that beta-FNA acts irreversibly at the mu receptor but reversibly at other receptors. Results similar to those with beta-FNA were obtained with Mr-1452, a drug previously reported to be relatively selective for kappa receptors. Thus, beta-FNA and Mr-1452, like naltrexone, appear to lack the receptor selectivity seen with ICI-174864.

ACKNOWLEDGEMENTS

This study was supported by USPHS grant DA-00254. Beta-FNA was provided by Dr. D. Zimmerman, LY-123502 by Dr. P. Gesellchen, and ICI-174864 by Drs. P. James and R. Cotton.

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