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STUDIES IN VITRO WITH ICI 174,864, [D-Pen^2, D-Pen^5]-ENKEPHALIN (DPDPE) AND [D-Ala^2, NMePhe^4, Gly-ol]-ENKEPHALIN (DAGO)

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ABSTRACT

The interactions of a proposed, selective delta receptor antagonist (ICI 174,864) and selective agonists at mu and delta receptors, [D-Ala^2, NMePhe^4, Gly-ol]-enkephalin (DAGO) and [D-Pen^2, D-Pen^5]-enkephalin (DPDPE), respectively, have been studied using the electrically-stimulated mouse isolated vas deferens (MVD) and the guinea-pig isolated ileum (GPI). Incubation of increasing concentrations of ICI 174,864 (10,30,100 and 300 nM) produced a dose-related and parallel rightward displacement of the DPDPE dose-response curve in the MVD. In contrast, ICI 174,864 (300-3000 nM) failed to affect the DAGO dose-response curve in the same tissue. Analysis of the DPDPE-ICI 174,864 interaction in the MVD using the pA_2 method revealed a Schild plot slope of -0.68 suggesting the involvement of more than one population of receptors. ICI 174,864 (300 nM) failed to antagonize DPDPE in the GPI at doses up to 30 uM. These results suggest that (a) ICI 174,864 acts as a selective delta antagonist in the MVD; (b) DPDPE interacts with mu receptors in the MVD but only at very high concentrations, and (c) delta receptors appear not to be of functional importance in the GPI.

INTRODUCTION

The existence of multiple opioid receptor subtypes has been postulated based in part on extensive work in vitro using the electrically stimulated guinea-pig isolated ileum (GPI) and the mouse vas deferens (MVD)(1). The importance of these in vitro bioassays may be viewed in part, as a basis for predicting the actions of opioids in vivo. Agonists which demonstrate a high degree of preference for the specific receptor subtypes in these tissues have been developed. These selective agonists include [D-Ala^2, NMePhe^4, Gly-ol]-enkephalin (DAGO) for the mu receptor (2) and cyclic [D-Pen^2, D-Pen^5]-enkephalin (DPDPE) for the delta receptor (3). Recently, ICI 174,864 [Allyl$_2$-Tyr-Aib-Aib-Phe-Leu-OH], has been reported to be a compound with selective antagonist properties at the delta receptor (4). The present work investigated the selectivity profile of ICI 174,864 in the MVD using the selective delta and mu agonists, DPDPE and DAGO, respectively. Further, the ability of ICI 174,864 to antagonize DPDPE in the GPI was studied in an attempt to determine whether DPDPE interacts with delta receptors in this tissue.

METHODS

Strips of longitudinal muscle-myenteric plexus were taken from Hartley guinea-pigs of either sex (300-400 g) and suspended in Krebs-bicarbonate buffer at 37° C in an organ bath. Similarly, vasa deferentia from male, ICR mice (25-30 g) were removed and suspended in magnesium-free Krebs-bicarbonate buffer. The tissues were transmurally stimulated with supramaximal pulses (0.1 Hz, 20 V, 0.4 msec, GPI; 0.1 Hz, 20 V, 2 msec, MVD) as previously described (3). Agonists were placed in the bath and the twitch height was measured after 3 min. The antagonist was added to the bath 2 min prior to the agonist, with 8 min being allowed between successive doses of agonist.

RESULTS

ICI 174,864 displaced the DPDPE dose-response curve to the right in a dose-related and parallel fashion in the MVD (fig. 1). In contrast, ICI 174,864 failed to displace the DAGO dose-response curve to the right in the same tissue (fig. 2). In the GPI, DPDPE acted as a full agonist, though at doses 1000 fold higher than those necessary for activity in the MVD. The IC_{50} for DPDPE in the GPI was calculated to be 14.5 (8.3 - 25.4) μM. ICI 174,864 failed to antagonize DPDPE in the GPI at a dose (300 nM) that effectively blocked the effects of this agonist in the MVD (fig. 3).

Analysis of the ICI 174,864-DPDPE interaction in the MVD using the pA_2 approach revealed a linear Schild plot ($r = 0.98$) with a slope of -0.687 and a pA_2 of 8.23 (8.09-8.37).

DISCUSSION

The present study has attempted to determine the selectivity profile of ICI 174,864 in vitro using the most selective agonists at mu (DAGO) and delta (DPDPE) receptors currently available. ICI 174,864 inhibited the effect of DPDPE, but not that of DAGO, in the MVD, indicating a selective interaction of the antagonist with delta receptors. However, when the ICI 174,864-DPDPE pA_2 was determined, the slope of the Schild plot was found to differ from the theoretically predicted unity. This deviation from theory may be explained on the basis of a non-preferential interaction of DPDPE with mu receptors, known to be present in the MVD. The observation of a Schild slope differing from unity has been previously reported (4) using ICI 174,864 and [D-Thr^2, Leu^5, Thr^6]-enkephalin (DTLET). These investigators showed that the Schild slope reverted to unity when the tissues studied were taken from C57BL/6 mice, a species previously found to be lacking in mu receptors (5).

In contrast to the activity of ICI 174,864 in atagonizing DPDPE in the MVD, this compound was ineffective against the delta agonist in the GPI. This finding suggests that DPDPE is interacting with mu, rather than delta, receptors in this tissue. It should be noted that a high dose of DPDPE is required for activity (30 μM) in the GPI and even a high dose of ICI 174,864 (300 nM) was not effective, adding to the belief of mu receptor involvement. The presence of functional delta receptors in the GPI is controversial. Using the non-equilibrium opioid antagonist, beta-chlornaltrexamine, some investigators have suggested that delta receptors are present in the ileum (6). While it remains unclear whether delta receptors are located in the

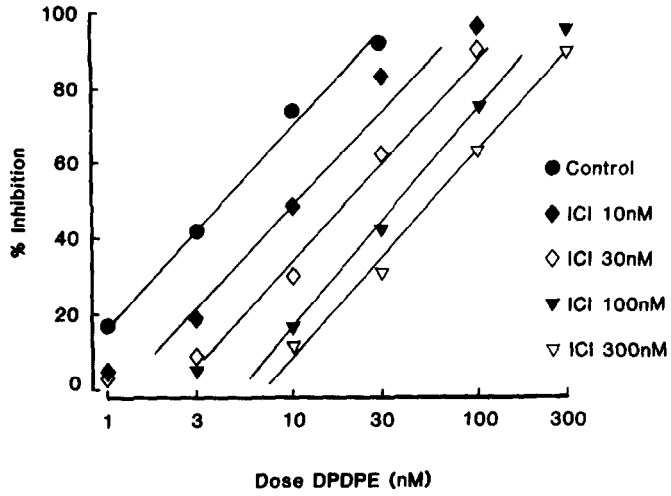
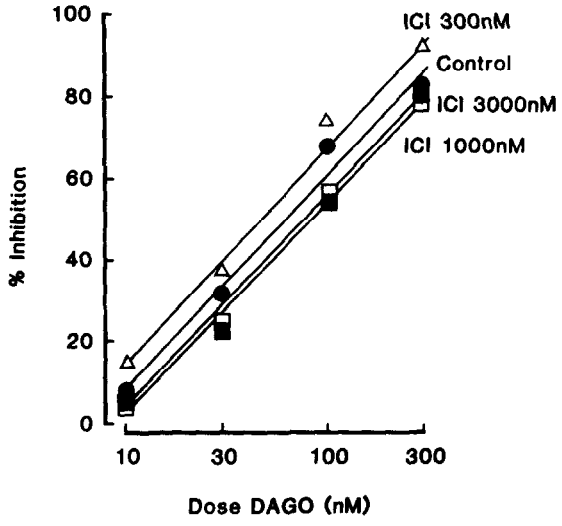


Figure 1. Interaction of increasing concentrations of ICI 174,864 and DPDPE in the mouse vas deferens.



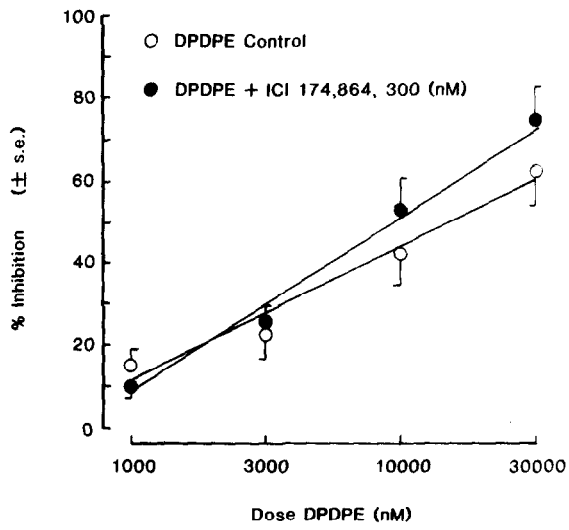


Figure 2 (left). Interaction of increasing concentrations of ICI 174,864 and DAGO in the mouse vas deferens.

Figure 3 (right). Interaction of DPDPE with ICI 174,864 (300 nM) in the GPI.

GPI, the present results suggest that delta receptors are of little functiona importance in this tissue.

The present work supports the concept of ICI 174,864 as a selective delta antagonist in vitro. However, the profile of this compound in vivo as reported in the current Proceedings (7,8) suggests only a partially-selective antagonist with a narrow window of activity. Furthermore, agonist effects of ICI 174,864 were also observed, suggesting that the profile of agonists and antagonists in bioassays must be viewed with caution. The usefulness of ICI 174,864 in other assays in vitro remains to be determined.

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