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Bremazocine differentially antagonizes responses to selective μ and δ opioid receptor agonists in rat hippocampus

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1 The effects of μ, δ and κ opioid receptor agonists were examined on evoked field potentials in brain slices prepared from rat hippocampus.

2 The effects of the μ-selective opioid peptide [D-Ala^2, NMe-Phe^4, Met(O)^{5}ol]enkephalin (FK 33-824) and the δ-selective peptide [D-Pen^2, D-Pen^5]enkephalin (DPDPE) were qualitatively and quantitatively similar. Both increased the amplitude of evoked population spike responses when perfused in low nanomolar concentrations in a fashion consistent with what has been previously reported for other opiate agonists such as morphine. The κ-selective agonists bremazocine and U-50, 488H were without effect upon evoked responses at concentrations as high as 10 μM.

3 Bremazocine, but not U-50, 488H, proved to be an extremely potent antagonist of responses to both μ- and δ- selective agonists. Moreover, bremazocine was considerably more potent in antagonizing responses to FK 33-824 than DPDPE, which supports the hypothesis that FK 33-824 and DPDPE act via different receptors. Thus, although bremazocine is an agonist at κ receptors, it appears to act as an antagonist at other opioid receptor sites.

Introduction

Evidence from both functional as well as ligand binding studies has supported the hypothesis that the effects of opioid drugs on the central nervous system are mediated via multiple receptors (Kosterlitz, 1985). The development of highly selective ligands for opioid receptor subtypes has facilitated attempts to identify the receptors mediating specific opioid-induced responses. Although some controversy remains regarding the proper categorisation of naloxone-sensitive receptors, the available evidence strongly supports the existence of a putative μ receptor, for which morphine is the prototypic ligand, a δ receptor, which has a relative selectivity for peptides such as methionine and leucine enkephalin, and a κ receptor, for which dynorphin A and related peptides appear to be the endogenous ligands. Although morphine and the opioid peptides are somewhat selective for the different receptor subtypes, the degree of such selectivity is often insufficient to permit the identification of the receptor involved in specific physiological responses. An additional complication from a physiological standpoint is that multiple receptor subtypes can often be found in the same brain region, and even on the same neurones (Williams & Zieglgänsberger, 1981; Werz & Macdonald, 1983).

In the central nervous system, considerable interest has focused on the actions of opioids in the hippocampus; in this brain region, morphine and opioid peptides have an excitatory effect that reflects the ability of opioids to inhibit the firing of inhibitory interneurones (Zieglgänsberger *et al.*, 1979; Dunwiddie *et al.*, 1980; Lee *et al.*, 1980; Nicoll *et al.*, 1980; Robinson & Deadwyler, 1980). Despite considerable work in this area, it is somewhat unclear as to what receptors mediate these excitatory responses. Peptides such as [D-Ala^2-D-Leu^5]enkephalin, which have a modest degree of selectivity for δ receptors in binding assays, are quite active (EC_{50} ~100 nM). Although several μ-selective peptides, such as [D-Ala^2, NMe-Phe^4, Met(O)^{5}ol]enkephalin (FK 33–824) and morphiceptin, exhibit relatively high potencies (Gähwiler & Maurer, 1981; Bostock *et al.*, 1984), morphine itself is a relatively weak agonist (EC_{50} ~3 μM; Robinson & Deadwyler, 1980; Dingledine, 1981; Valentino &

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Dingledine, 1982).

In terms of the effects of κ-selective agonists, the picture is even less clear. Dynorphin A (1–17) in subnanomolar concentrations can increase the population spike response (as do μ- and δ-agonists), but can also decrease the evoked response in higher concentrations via a naloxone-insensitive mechanism (Vidal *et al.*, 1984); somewhat similar effects have been reported with other κ agonists as well (Brookes & Bradley, 1984). Bremazocine, another κ agonist, has little effect by itself on cultured hippocampal neurones except in micromolar concentrations, but antagonizes the excitatory effects of the μ-selective agonist FK 33-824 (Gähwiler & Maurer, 1981). Ethylketocyclazocine, the prototypic κ ligand, has been reported to have little or no activity as either an agonist or an antagonist in the hippocampal slice preparation (Valentino & Dingledine, 1982), but has weak antagonist properties on cultured hippocampal neurones (Gähwiler & Maurer, 1981).

The difficulties in determining which receptors mediate specific responses stems partially from the fact that many of the drugs tested have only limited selectivity for the different receptor subtypes. However, the recent development of peptides possessing a high degree of selectivity raises the possibility of improving the characterization of the opioid receptor subtypes that mediate physiological responses in the hippocampus. An additional problem with many of the previous studies stems from the fact that nearly all comparisons have been made between selective agonists. There are a variety of reasons why estimates of the affinities of agonists determined from binding studies may not accurately reflect agonist potencies, such as would be measured in physiological studies; such factors may include different affinity states of the receptor, differences in the efficacy of agonists, development of tolerance, spare receptors, etc. For these reasons, it is usually preferable to use antagonist affinities when attempting to differentiate and characterize multiple receptor subtypes (North, 1986). Although naloxone appears somewhat μ-selective (Kosterlitz, 1985), previous studies in the hippocampus have suggested that it has little or no ability to discriminate between responses to μ- and δ-selective agonists (Valentino & Dingledine, 1982; Neumaier *et al.*, 1986). Such investigations would be facilitated by the use of antagonists with a higher degree of receptor selectivity.

In order to characterize the pharmacology of hippocampal opioid responses in greater detail, we have taken advantage of an agonist with relative selectivity for the μ receptor (FK 33-824; Roemer *et al.*, 1977; Chang & Cuatrecasas, 1979), and a highly δ-selective peptide, [D-Pen^2, D-Pen^5]enkephalin (DPDPE; Mosberg *et al.*, 1983). In addition, we have characterized the antagonist potencies of the κ-selective opioid agonists bremazocine and U-50, 488H against these selective agonists. Bremazocine has been reported to antagonize μ and δ opioid responses in the rat vas deferens, and to show some selectivity for the μ-receptor (Gillan *et al.*, 1981). Bremazocine can also antagonize morphine analgesia, whereas U-50, 488H does not generally appear to exhibit opioid antagonism (Lahti *et al.*, 1982; Von Voigtlander & Lewis, 1982). For this reason, we have characterized responses to both U-50, 488H and bremazocine in the rat hippocampal slice preparation in order to determine whether multiple opioid receptors participate in responses to different agonists, and whether antagonistic effects at μ- and δ-receptors are general properties of κ-receptor agonists in this system.

Methods

Male Sprague-Dawley rats weighing between 150–250 g were obtained from Sasco (Omaha, Nebraska, U.S.A.). Hippocampal brain slices were prepared as described previously (Dunwiddie & Lynch, 1978; Yasuda *et al.*, 1986) and immediately placed in chilled medium containing (mM) NaCl 124, KCl 4.9, KH_2PO_4 1.2, $MgSO_4$ 2.4, $CaCl_2$ 2.5, $NaHCO_3$ 25.6 and glucose 10 (pH 7.5) that was pregassed with 95% O_2 and 5% CO_2. Slices were transferred to a recording chamber maintained at 33–34°C, and perfused during the recording period with a constant flow of fresh oxygenated preheated medium at a rate of 2 ml min^{-1}.

A total of 132 slices from 38 rats were examined. Twisted nichrome wire stimulation electrodes were placed under visual guidance in the stratum radiatum near the border of CA1-CA2, and monosynaptic responses were evoked in CA1 by stimulation with monophasic 0.1 ms pulses of 6–30 V delivered to the slice at 1 min intervals. In every slice, the voltage was set so that the negative-going population spike response was approximately equal in magnitude to the amplitude of the positive field potential upon which it was superimposed. Evoked potentials were recorded with 2–3 MΩ glass electrodes filled with 3 M NaCl placed in the CA1 region under visual guidance. Responses were recorded from the CA1 pyramidal cell layer, and analysed on-line by a NOVA 3/12 computer. Drugs were made up in degassed deionized water at 100–1000 times the desired final concentration, then added to the flow of perfusion fluid with a calibrated syringe pump.

Slices were initially tested with a concentration of agonist that, on the basis of preliminary studies, was sufficient to evoke at least a 95% of maximal increase in the population spike response; the response to that concentration of drug was defined as the maximal response for that particular slice. Subsequently, the same slice was then tested with different concentra-

tions of agonists and/or antagonists, and all subsequent responses expressed as a percentage of the initial test response. Moderate changes in the baseline between the pre- and post-drug periods were corrected for by the procedure illustrated in Figure 3. Slices that manifested a high degree of shift in the baseline were not used. The potential degree of interference with these measures due to the development of tolerance during the initial test period was assessed by conducting control experiments in which the slice was tested repeatedly with the same concentration of agonist. Although slices tested with FK 33-824 did not show any tolerance according to this procedure, those tested with DPDPE showed a modest degree of tolerance with extended perfusion (as much as a 50% drop in the response during a 1 h perfusion). For this reason, the test periods with DPDPE were kept relatively brief, so that the maximum reduction in the second test response due to tolerance was less than 10%. When the effects of antagonists were examined on DPDPE responses, such responses were always compared to appropriate controls in which slices were perfused more than once with DPDPE without any antagonist present.

Hill plots of the raw data were used for the calculation of EC_{50} values, which correspond to the concentrations of agonist required to elicit 50% of maximal increases in the population spike response amplitude (Dunwiddie & Fredholm, 1984), and the associated 95% confidence limits. *Estimated* K_e values for bremazocine were determined from the relation $K_e = ([B]*[A])/([A'] - [A])$, where [B] represents the concentration of bremazocine in the perfusion medium, and [A] and [A'] represent the concentrations of agonist required to elicit equivalent responses in the absence and presence of bremazocine respectively. The extent to which these estimated values correspond to the actual equilibrium dissociation constants will reflect both the extent to which equilibrium conditions can be achieved in the brain slice, and the validity of the assumption that these drugs interact in a competitive manner.

Drugs

The drugs used were obtained from the following sources: FK 33-824, Sandoz; U-50, 488H (*trans*-(±)-3,4-dichloro-N-methyl-N-(2-(1-pyrrolidinyl)-cyclohexyl) benzeneacetamide, methanesulphonate hydrate), Upjohn Co.; [D-Pen^2, D-Pen^5]enkephalin, Peninsula Laboratories; bremazocine, ACF Chemiefarma.

Results

In previous papers, we have characterized the ability of agonists such as [D-Ala^2-Met^5]enkephalinamide to increase the magnitude of the evoked population spike response in rat hippocampal pyramidal cells without altering the evoked extracellular field e.p.s.p. response (Dunwiddie *et al.*, 1980), and demonstrated that this is an opioid-specific response. In the present experiments, we found that perfusion of hippocampal slices with either the μ-selective agonist FK 33-824 or the δ-selective agonist DPDPE resulted in marked increases in the population spike response that were qualitatively indistinguishable from each other. An example of a response to FK 33-824 is illustrated in Figure 1. The thresholds for responses to both FK 33-824 and DPDPE were approximately 5 nM; the EC_{50} values calculated from Hill plots of the dose-response curves were 13 nM and 14 nM respectively. The maximal increase in the population spike response that was observed for both agonists was also not significantly different; DPDPE induced an average 185 ± 19% increase in the population spike response ($n = 36$), whereas FK 33-824 elicited an increase of 217 ± 22% ($n = 34$; Student's t test = 1.10, $P > 0.10$). These results are comparable to those previously obtained with [D-Ala^2-Met]enkephalinamide, an agonist with relatively little selectivity for opioid receptor subtypes (Dunwiddie *et al.*, 1980; 1982). The only significant difference that was observed between responses to DPDPE and FK 33-824 was that responses to DPDPE showed significant decrement during prolonged perfusion, whereas responses to FK 33-824 did not.

Although both the μ- and δ-selective agonists elicited marked increases in the evoked population spike response, perfusion of slices with κ-selective agonists had no significant effect upon the evoked response. Slices were perfused for 10 min with 30 nM, 1 μM, and 10 μM bremazocine, and at the end of the test periods the mean ± s.e.mean population spike response as a percentage of the pre-drug control response was 93 ± 16, 99 ± 7.5, and 98 ± 13, respectively ($n = 3$, 6, and 6). U-50, 488H, another selective κ-agonist, was also without effect upon the population spike amplitude (mean percentage of control response following 1 μM was 98 ± 8.4, $n = 9$; response to 10 μM was 99 ± 11, $n = 6$). None of these values was significantly different from control slices untreated with drug ($P > 0.1$, two-tailed t test). Although bremazocine had no direct effect in these concentrations, it strongly antagonized excitatory responses to FK 33-824, DPDPE, morphine, and [D-Ala^2-Met]enkephalinamide, even in very low concentrations. When slices were first perfused with any of these agonists, increases in population spike amplitude were observed that could be rapidly reversed by subsequent addition of bremazocine to the perfusion medium (Figure 2). In addition, when slices were pretreated with bremazocine, previously effective concentrations of agonists no longer induced changes in population

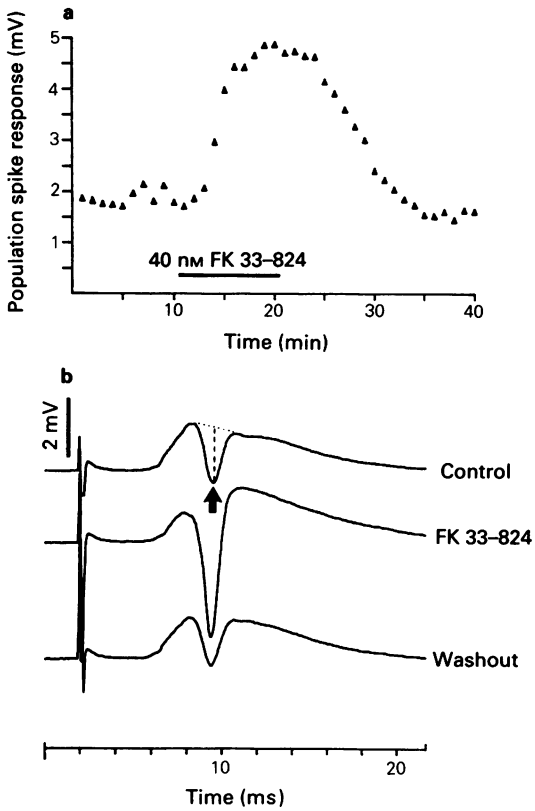

Figure 1 Opiate-mediated increase in population spike amplitude: (a) The amplitude of the population spike response, evoked at a frequency of 1 min^{-1}, is illustrated before and during perfusion with 40 nM FK 33-824. Although not shown, the increases induced by FK 33-824 in the population spike amplitude could be maintained for at least 30–60 min during continuous perfusion with drug. Similar increases in population spike amplitude were observed with DPDPE, but usually showed a slow diminution during extended superfusion. (b) Averaged evoked responses from the experiment shown in (a) during the pre-drug control period, during perfusion with 40 nM FK 33-824, and following drug washout. Each record is the average of 5 responses. The amplitude of the negative-going population spike (arrow) was determined as the length of the vertical dashed line illustrated for the control record.

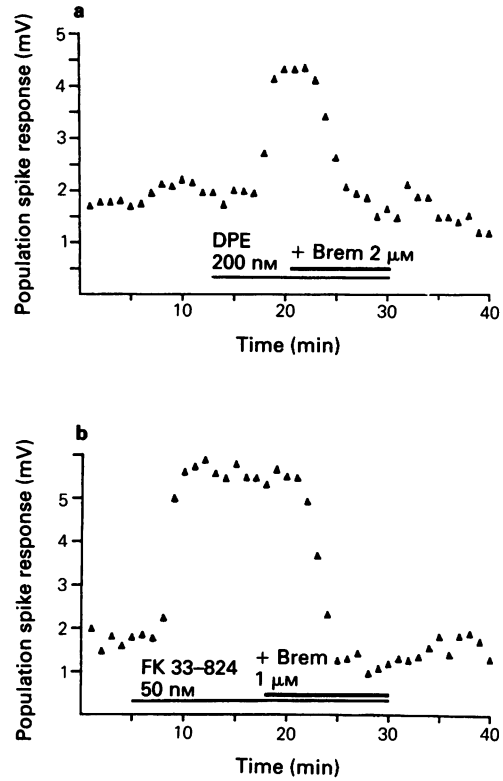

Figure 2 Bremazocine antagonism of opiate responses: (a) Perfusion of a single slice with a supra-maximal concentration of DPDPE (DPE; 200 nM) resulted in an approximate doubling of the population spike amplitude; this response was completely reversed by subsequent addition of 2 μM bremazocine (Brem) to the perfusion medium. (b) The antagonistic effect of 1 μM bremazocine on the response to a supra-maximal concentration of FK 33-824 (50 nM) is shown. In these initial experiments, high concentrations of bremazocine were used; however, in subsequent experiments, it was found that concentrations of bremazocine as much as 2–3 orders of magnitude lower could exert similar antagonistic effects (cf. Figure 3).

spike amplitude (data not shown). Although marked antagonistic effects were observed with bremazocine, the κ-agonist U-50, 488H did not appear to have corresponding antagonistic properties. Pretreatment of slices with a relatively high concentration of U-50, 488H (1 μM) had no significant effect upon responses to 20 nM FK 33-824 (mean response 99 ± 7.2% of control; $n = 6$; $P > 0.1$), and only partially reduced the responses to DPDPE (mean response 75 ± 4.8% of controls, $n = 11$; $P < 0.01$).

In order to characterize the mechanism underlying the antagonistic properties of bremazocine in greater detail, dose-response curves for the selective μ- and δ-agonists were determined, and the ability of bremazocine to induce shifts in the curves was examined. The protocol that was followed in these experiments is detailed in Figure 3. Slices were initially tested with a sufficient concentration of agonist in

order to determine the maximal opioid response for that slice, and the drug was washed out until the response amplitude returned to baseline. Subsequently, the slice was retested with increasing concentrations of agonist either in the presence or absence of bremazocine. The concentrations of bremazocine that were tested were determined on the basis of preliminary experiments to be those that induced an approximate 10 fold rightward shift in the dose-response relation. The results of these experiments are illustrated in Figure 4. Bremazocine significantly shifted the EC_{50} values for both FK 33-824 and DPDPE, without affecting the maximal response amplitude. However, the concentration of bremazocine required to shift the response to FK 33-824 was clearly much lower than that needed to induce a similar displacement in the curve for DPDPE. The apparent K_e values for bremazocine, calculated from the shift in the parallel portions of the dose-response curves, were 2.3 nM when DPDPE was used as the agonist, and 0.14 nM for FK 33-824 (Table 1). The dose-response curve for FK 33-824 but not DPDPE appeared to be flattened by bremazocine (Figure 4), and this is confirmed by the significantly lower slope calculated for FK 33-824 + bremazocine (Table 1).

Discussion and Conclusions

Previous workers have concluded that the excitatory actions of opioids in the hippocampus, as manifested by increases in the evoked population spike response, might be mediated via actions at both μ- and δ-opioid receptors (Bostock *et al.*, 1984). However, the selective agonists that previously have been available, such as [D-Ala^2-D-Leu^5]enkephalin (DADLE), had such low selectivity (in the case of DADLE, less than 7 fold for μ vs. δ) that it has been difficult to determine which receptors are involved. Nevertheless, several aspects of the present results confirm these previous conclusions. First, both FK 33-824 and DPDPE were found to be highly potent agonists in the hippocampal slice preparation, and their EC_{50} values corresponded roughly to expected values based upon previous ligand binding and displacement studies (Kosterlitz & Paterson, 1980; Kosterlitz, 1985). In accordance with

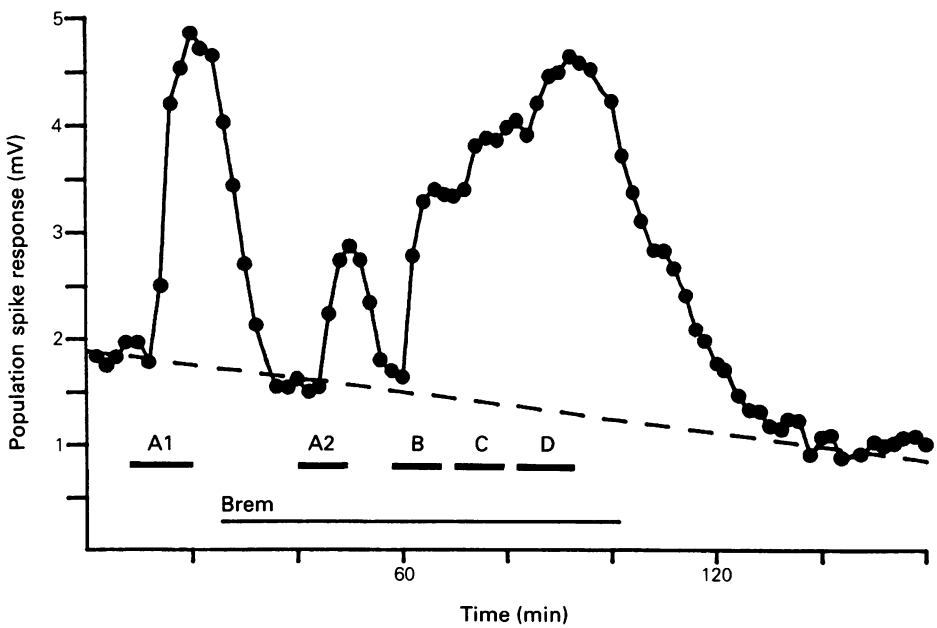

Figure 3 Bremazocine antagonism of responses to FK 33-824 in a single slice. The changes induced by FK 33-824 with and without bremazocine in the population spike response evoked from a single slice are shown. Perfusion with 40 nM FK 33-824 (denoted A1) was used initially to define the maximal drug response. The slice was then perfused with 0.5 nM bremazocine (Brem), and subsequently tested with 40 nM (A2), 200 nM (B), 1000 nM (C), and 5000 nM (D) FK 33-824. The response to 40 nM FK 33-824 in the presence of bremazocine was markedly reduced, and significantly higher concentrations were required to elicit the maximal response. Control dose-response curves were determined in a similar fashion but without bremazocine in the perfusion medium. The dashed line connecting the initial baseline to the end of the final washout period was used to estimate changes in the baseline response during the extended test period. All drug-induced responses were measured in relation to this estimated baseline.

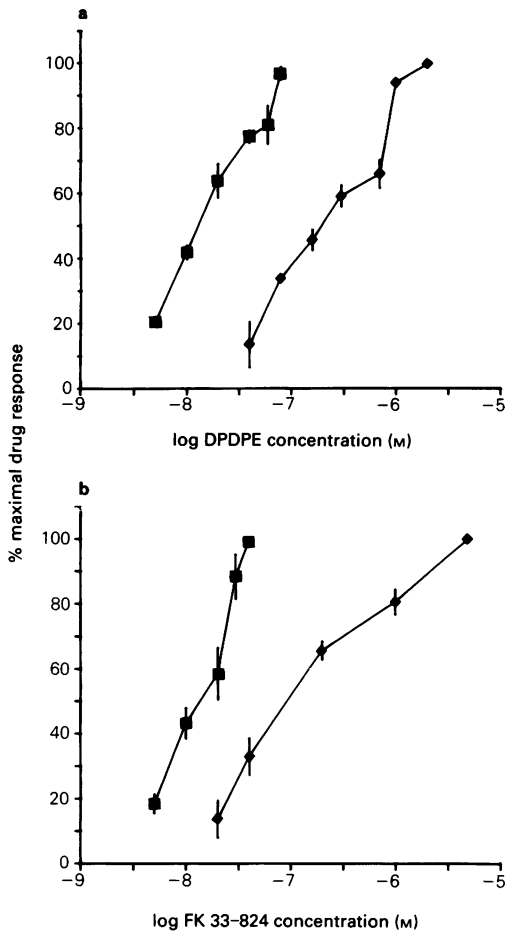

Figure 4 Effect of bremazocine on log dose-response curves. Dose-response curves for (a) DPDPE and (b) FK 33-824 derived from data obtained as in Figure 3, are illustrated: (■) control dose-response relation; (◆) data obtained in the presence of either 30 nM bremazocine (a) or 0.5 nM bremazocine (b). Each point on the curve represents the mean drug response as a percentage of the maximal drug response determined at the beginning of each experiment, thus, any shift in the maximal response due to the effects of bremazocine would be apparent; vertical lines show s.e.mean. The number of slices tested at each concentration ranged from 3–11, and were obtained from 2–5 animals. The estimates of the EC_{50} values and slopes for the agonists, and K_e values for bremazocine based upon these curves, are given in Table 1.

previous reports that δ- but not μ-receptors show a rapid down-regulation in hippocampal slices (Dingledine *et al.*, 1983), there also appeared to be a decrement in the response to the δ-selective agonist DPDPE with continued superfusion, but not to the μ-selective FK 33-824 compound, suggesting that different receptors mediate responses to the two agonists. Probably the most compelling evidence in support of the hypothesis that multiple receptors are involved is the observation that the interactions between bremazocine and DPDPE and FK 33-824 clearly differed in several respects. First, the apparent dissociation constants for bremazocine were significantly different for the two agonists. The extent to which these estimates reflect the actual K_e values rests upon the assumption that equilibrium conditions have been reached, and that the interaction between bremazocine and the agonists is competitive. Although it is difficult to determine whether an antagonist is at equilibrium, the time course of the effect of bremazocine (Figure 2) suggests that by the time agonist responses were measured (usually >20 min after the onset of bremazocine perfusion), bremazocine should be well-equilibrated thoughout the tissue. In any case, this would be unlikely to lead to systematic differences between the two agonists. Whether or not the antagonism is competitive is also difficult to determine; the change in the slope for the FK 33-824 dose-response curve but not for DPDPE suggests that in the former case, the interaction may not be competitive. If this is the case, then the estimated K_e value clearly would not be accurate, but the conclusion (i.e., that the receptors acted upon by the agonists are different) would have to be the same. The apparent K_e values that we have observed are similar (relatively selective for the μ-agonist) to what have been previously reported in rat vas deferens, (Gillan *et al.*, 1981), but the absolute values are somewhat lower, and the degree of selectivity greater than was observed.

The change in the slope of the dose-response relation for FK 33-824 in the presence of bremazocine that was observed could arise from a variety of mechanisms (e.g., partial agonist effects, non-competitive antagonism, etc.). Another possibility is that the FK 33-824 analogue, which is considerably less selective than DPDPE (approximately 10–25 fold selectivity for the μ-receptor; Chang *et al.*, 1979; Kosterlitz & Paterson, 1980), acts upon both μ- and δ-receptors, and the resultant dose-response curve is a composite of the two responses. Our data suggest that the interaction of bremazocine with the receptor upon which DPDPE acts is of a simple competitive nature, but the type of interaction between bremazocine and FK 33-824 remains unclear.

An unusual aspect of the opioid pharmacology of the hippocampus is the relatively low potency of morphine and related alkaloids. We have found the μ-selective peptide FK 33-824 to be highly active in hippocampus ($EC_{50} = 13$ nM), and Bostock *et al.* (1984) reported the μ-selective morphiceptin analogue

Table 1 Analysis of [D-Ala^2, NMe-Phe^4, Met(O)^{5}ol]enkephalin (FK 33-824) and [D-Pen^2, D-Pen^5]enkephalin (DPDPE) dose-response curves

Treatment	*ED_{50}* (nM)	*95% conf. limits* (nM)	*Hill slope*	n	*Correlation coefficient*	*K_e Brem* (nM)
DPDPE	14	12–15	1.5	42	0.95	
DPDPE + bremazocine 30 nM	190	160–230	1.2	36	0.91	2.3 (1.7–3.2)
FK 33-824	13	11–16	1.5	31	0.82	
FK 33-824 + bremazocine 0.5 nM	120	80–170	0.75†	26	0.89	0.14 (0.065–0.38)*

† Significantly different from slopes for other 3 curves (no overlap with 95% confidence limits)
*K_e calculated from the EC_{20} (rather than EC_{50} value) because of slope change in upper part of dose range (see Figure 4). The K_e determined from the EC_{50} values was 0.064 nM (95% limits 0.035–0.12 nM)

[N-MePhe^3-DPro^4]morphiceptin (PL017) to be quite active as well. Morphine, on the other hand, is at least 100 fold less potent than the μ-selective peptides, with an EC_{50} value of approximately 3.0 μM (Robinson & Deadwyler, 1980; Bostock *et al.*, 1984). This does not appear to correspond at all to relative affinities of these drugs for putative opioid receptors in brain; for example, Chang *et al.* (1979) have reported that morphine is approximately 3 times *more* potent than FK 33-824 in displacing ^{125}I-labelled FK 33-824 from binding sites in rat brain membranes. Similarly, the opioid antagonist naloxone has a relatively high affinity for opioid receptors in some tissues (e.g., K_e of 2 nM in the locus coeruleus *in vitro*; Williams & North, 1984), whereas in the hippocampus, we have previously estimated the K_e value for naloxone to be approximately 130 nM (Dunwiddie *et al.*, 1982). Moreover, Valentino & Dingledine (1982) have suggested that naloxone is if anything more potent in antagonizing the effects of [D-Ala^2-D-Leu^5]enkephalin than morphine, whereas in most preparations naloxone has a weak selectivity for μ-vs. δ-receptors. Taken together, these results may suggest that the opiate receptor upon which μ-selective peptides act in this preparation differs somewhat from the 'classical' μ-receptor found in such tissues as the guinea-pig ileum or rat vas deferens.

Although bremazocine is clearly a potent antagonist of the effects of μ- and δ-selective peptides in hippocampus, the present results suggest that this is unlikely to be a general property of κ-agonists. At the receptor upon which FK 33-824 exerts its primary actions, U-50, 488H did not appear to have any antagonistic effects even at a concentration of 1 μM. If bremazocine exerts antagonistic effects as a direct consequence of occupancy at a κ-receptor, then based upon the relative affinities of bremazocine and U-50, 488H for κ-receptors (Kosterlitz, 1985), one would expect U-50, 488H to be approximately a 10 fold weaker antagonist than bremazocine; this was clearly not the case. In addition, if one κ-agonist (bremazocine) was a μ-selective antagonist, and if this is an intrinsic property of all agonists that can occupy the κ-receptor, then one would expect all κ-agonists to show such selectivity. The fact that U-50, 488H was if anything a δ-selective antagonist is again inconsistent with this being a κ-agonist-related effect.

The lack of direct actions of both bremazocine and U-50, 488H in the CA1 region of hippocampus suggests that whatever the role of the κ-receptor in hippocampus, the changes it elicits in cellular function are sufficiently subtle that they do not result in direct alterations in evoked population spike amplitude. On the other hand, these experiments provide strong support for the idea that multiple (perhaps μ- and δ-) opioid receptors can induce increases in the evoked population spike response in hippocampus. Although the present studies do not indicate whether or not they do so via the same mechanisms or effects on the same cell types, there are numerous precedents for μ- and δ-receptors exerting parallel actions (Williams & Zieglgänsberger, 1981; Werz & Macdonald, 1983). The relative selectivity of bremazocine, which appears to differentiate between the receptors upon which DPDPE and FK 33-824 act, suggests that it may be an effective tool in characterizing subtypes in multiple opioid receptor systems.

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