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Pharmacological profile of various κ-agonists at κ-, μ- and δ-opioid receptors mediating presynaptic inhibition of neurotransmitter release in the rat brain

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1 The potency, relative efficacy and selectivity of a series of κ-opioid receptor agonists at the μ-, δ- and κ-opioid receptors mediating inhibition of electrically-induced (radiolabelled) neurotransmitter release from superfused rat brain slices was determined.

2 With regard to their potencies at κ-receptors mediating inhibition of striatal [^{3}H]-dopamine release, the highest pD_2 value (8.7) was found for bremazocine and the lowest (7.1) for U50488; the pD_2 values for ethylketocyclazocine (EKC), tifluadom, U69593 and PD117302 were between 8.0 and 8.3. There were no marked differences between the relative efficacies of the κ-agonists (maximum inhibition being 60–70%). In contrast to the other κ-agonists, at a concentration of 1 μM, PD117302 caused a significant (25–40%) increase of the spontaneous efflux of tritium.

3 None of the κ-agonists significantly affected striatal [^{14}C]-acetylcholine (ACh) release, with the exception of a slight inhibitory effect of EKC. The δ-receptor-mediated inhibitory effect of [D-Ala^2, D-Leu^5] enkephalin (DADLE) on [^{14}C]-ACh release was antagonized in a concentration-dependent manner by bremazocine (0.1 and 1.0 μM) and also partially by EKC (1 μM), but not by the other κ-agonists. The pA_2 value for bremazocine as an antagonist at the δ-receptors involved was 8.0, compared to 7.6 for naloxone.

4 None of the κ-agonists significantly affected cortical [^{3}H]-noradrenaline (NA) release, with the notable exception of tifluadom, which strongly inhibited release by activating μ-receptors. The μ-receptor-mediated inhibitory effect of Tyr-D-Ala-Gly-(NMe)Phe-Gly-ol (DAMGO) on [^{3}H]-NA release was antagonized in a concentration-dependent manner by bremazocine and EKC, but not by the other κ-agonists. The pA_2 value for bremazocine as an antagonist at the μ-receptors involved was 8.2, compared to 8.6 for naloxone.

5 Thus, whereas U69593 and PD117302 display high potency and selectivity towards κ-opioid receptors, the potent benzomorphan κ-agonists bremazocine and EKC also appear to be strong μ-opioid receptor antagonists.

Introduction

Both in peripheral tissues and in the central nervous system opioid receptors can be separated into at least three pharmacologically distinct types, μ-, δ- and κ-receptors (Wood, 1982; Paterson *et al.*, 1983; Martin, 1984; Goldstein & James, 1984; Zukin & Zukin, 1984). Previous studies on presynaptic modulation of neurotransmitter release from rat brain slices have shown that activation of different opioid receptor subtypes may have different functional consequences. Thus, dynorphin and some other agonists with κ-opioid receptor selectivity, such as bremazocine and U50488, were found to inhibit the depolarization-induced release of [^{3}H]-dopamine from rat striatal slices, with little or no effect on the release of [^{14}C]-acetylcholine (ACh) (Mulder *et al.*, 1984; 1989; Schoffelmeer *et al.*, 1988; Werling *et al.*, 1988). On the other hand, Leu-enkephalin and many of its analogues appear to inhibit striatal ACh release through activation of δ-opioid receptors, without affecting dopamine release (Mulder *et al.*, 1984; 1989; Schoffelmeer *et al.*, 1988). Finally, μ- but not δ- or κ-receptor activation results in an inhibition of [^{3}H]-noradrenaline (NA) release from rat cortical or hippocampal slices and synaptosomes (Mulder *et al.*, 1984; 1987; Jackisch *et al.*, 1986; Werling *et al.*, 1987; Schoffelmeer *et al.*, 1988).

In the present study we have determined the potency, relative efficacy and selectivity of a series of κ-agonists at different opioid receptors mediating inhibition of neurotransmitter release in various regions of the brain. The drugs examined were the benzomorphans bremazocine (Römer *et al.*, 1980) and ethylketocyclazocine (EKC), the benzodiazepine analogue tifluadom (Römer *et al.*, 1982) and the cyclohexyl benzeneacetamide derivatives U50488 (Von Voigtlander *et al.*, 1983), U69593 (Lahti *et al.*, 1985; Clark *et al.*, 1988a) and PD117302 (Leighton *et al.*, 1987; Clark *et al.*, 1988b). Previous studies, investigating opioid agonist-induced inhibition of neurally-evoked contractions in smooth muscle preparations have shown that, in addition to acting as κ-agonists, bremazocine and other benzomorphans may behave as δ- and/or μ-opioid receptor antagonists (Gillan *et al.*, 1981; McKnight *et al.*, 1985; Corbett & Kosterlitz, 1986; Miller *et al.*, 1986; Sheehan *et al.*, 1986; Takemori *et al.*, 1986). Ligand-receptor binding studies have revealed that bremazocine and other benzomorphan κ-opioid agonists display quite high affinities for δ- and/or μ-binding sites in the brain (Gillan & Kosterlitz, 1982; Paterson *et al.*, 1983). Furthermore, a neurophysiological study (Dunwiddie *et al.*, 1987) has demonstrated that bremazocine, but not U50488, is a potent antagonist at μ-opioid receptors in the rat hippocampus. Therefore, in the present study the different κ-agonists were examined for possible antagonist actions at μ- and δ-opioid receptors mediating presynaptic inhibition of neurotransmitter release.

Methods

Preparation and incubation of brain slices

Male Wistar rats (160–200 g body weight) were decapitated and the brains rapidly removed. Neocortical and/or neostriatal slices (approx. 0.3 × 0.3 × 2 mm) were prepared, labelled and superfused essentially as described previously (Stoof *et al.*, 1982; Mulder *et al.*, 1984; 1989; Schoffelmeer *et al.*, 1988). In

brief, neocortical slices (about 100 mg fresh tissue weight) were incubated for 15 min in 2.5 ml Krebs-Ringer-bicarbonate medium containing 0.1 μM [^{3}H]-NA to label noradrenergic nerve terminals selectively. Similarly, striatal slices were incubated in medium containing 0.1 μM [^{3}H]-dopamine and 1 μM [^{14}C]-choline, resulting in a selective labelling of dopaminergic and cholinergic nerve terminals, respectively.

Superfusion of brain slices and addition of drugs

After labelling the slices were transferred to each of 24 chambers (volume 0.2 ml; 3–4 mg of tissue per chamber) of a superfusion apparatus and subsequently superfused (0.25 ml min^{-1}) with medium (gassed with 95% O_2 : 5% CO_2) at 37°C. After 40 min of superfusion (i.e. t = 40 min) the superfusate was collected in 10 min samples.

At t = 50 min the slices were exposed to electrical stimulation (biphasic square wave pulses, 1 Hz, 2 ms duration) for 10 min to induce calcium-dependent release of the radiolabelled neurotransmitters. For [^{3}H]-NA release from neocortical slices a current of 15 mA was used and for [^{3}H]-dopamine and [^{14}C]-ACh release from striatal slices 30 mA.

When tested as agonists the drugs were added to the superfusion medium 10 min before electrical stimulation of the slices and remained present until the end of the experiment. When tested as antagonists at μ- and δ-receptors the κ-agonists were added to the medium 20 min before stimulation and the respective μ- and δ-agonists, i.e. Tyr-D-Ala-Gly-(NMe) Phe-Gly-ol (DAMGO; 1 μM) and [D-Ala^2, D-Leu^5]enkephalin (DADLE; 1 μM), were added 10 min later. In each experiment quadruplicate observations were made. At the end of the experiment the radioactivity remaining in the tissue was extracted with 0.1 M HCl. In one series of experiments (see Figure 2) the μ- and δ-antagonist actions of bremazocine were analyzed further by a cumulative dose-response technique, developed previously for studying the pharmacological characteristics of presynaptic receptors in superfused brain slices and described in detail elsewhere (Frankhuyzen *et al.*, 1982). In brief, cortical slices labelled with [^{3}H]-NA or striatal slices labelled with [^{3}H]-dopamine and [^{14}C]-choline were subjected to continuous electrical field stimulation (biphasic square wave pulses, 1 Hz, 2 ms duration) from 40 min after beginning the superfusion (t = 40 min). In experiments with cortical slices a current of 15 mA was used and the superfusion medium routinely contained the NA uptake inhibitor desipramine (3 μM). In experiments with striatal slices the stimulation current was 30 mA and the superfusion medium contained the dopamine uptake inhibitor nomifensine (1 μM). Increasing concentrations of the μ-agonist DAMGO and the δ/μ-agonist DADLE were added cumulatively to the medium at 10 min intervals, from t = 50 to t = 80 min, either in the absence or presence of bremazocine or naloxone added to the medium from t = 20 min. In each experiment the inhibitory effect of DAMGO or DADLE was determined (in duplicate), by comparing the stimulation-evoked release of radiolabelled transmitter in its presence with the release found in control superfusion chambers not exposed to the agonist.

Calculation of release data

The radioactivity in the superfusion samples and tissue extracts was determined by liquid scintillation counting. The efflux of radioactivity during each 10 min collection period was expressed as fractional release, i.e. as a fraction of the amount of radioactivity present in the tissue at the beginning of the respective collection period. To calculate the electrically-evoked release of the radiolabelled neurotransmitter, the spontaneous efflux of radioactivity was subtracted from the total overflow of radioactivity during stimulation and the subsequent 10 min. The spontaneous efflux of radioactivity from striatal slices labelled with [^{3}H]-dopamine and [^{14}C]-ACh was 0.25–0.30% min^{-1} (^{3}H) and 0.20–0.25% min^{-1} (^{14}C), respectively, of total tissue radioactivity; the spontaneous efflux of tritium from cortical slices labelled with [^{3}H]-NA was 0.15–0.20% min^{-1}.

The electrically-evoked release of radioactivity (in excess of spontaneous efflux) from striatal slices amounted to 3–4% ([^{3}H]-dopamine) and 6–8% ([^{14}C]-ACh), respectively, of total tissue content and from cortical slices labelled with [^{3}H]-NA, 3.5–4.5%.

A detailed description of the calculation of the data obtained in the experiments using the cumulative dose-response technique has been published elsewhere (Frankhuyzen *et al.*, 1982). In these experiments the spontaneous efflux of radioactivity was determined separately in chambers in which the slices were not exposed to electrical stimulation.

The concentration-effect curves, Hill slopes and pD_2-values ($pD_2 = -\log EC_{50}$) were derived from the release data by the non-linear curve-fitting programme ALLFIT. Statistical analysis of the data was carried out with a two-way analysis of variance, followed by Duncan's multiple range test, using SPSS/PC + V2.0 (SPSS, Inc.).

Radiochemicals and drugs

1-[7-^{3}H]-noradrenaline (37 Ci mmol^{-1}), [7,8-^{3}H]-dopamine (47 Ci mmol^{-1}) and [methyl-^{14}C]-choline (50 mCi mmol^{-1}) were purchased from the Radiochemical Centre (Amersham) and DAMGO and DADLE from Bachem. The following drugs were kindly donated: bremazocine and tifluadom by Sandoz, ethylketocyclazocine by Stirling-Winthrop, U50488 (*trans* - 3,4 -dichloro - N - methyl - N - [2 - (1 - pyrrolidinyl) - cyclohexyl]benzene-acetamide) and U69593 by (5α,7α,8β-(−)-N-methyl - N - [7 - (1 - pyrrolidinyl) - 1 - oxaspiro(4,5)dec - 8 - yl] phenyl-benzeneacetamide) Upjohn and PD117302 ((±)-trans - N - methyl - N[2 - (1 - pyrrolidinyl) - cyclohexyl]benzo[b] thiophene-4-acetamide) by Parke-Davis.

Results

Striatal [^{3}H]dopamine release: κ-receptor agonism

All of the κ-agonists inhibited the electrically-evoked release of [^{3}H]-dopamine from striatal slices (Figure 1). At a concentration of 1 μM the κ-agonists slightly decreased (by 10–15%) the spontaneous efflux of radioactivity, with the exception of PD117302, which increased spontaneous efflux by 25–40%.

The concentration-response curves shown in Figure 1 were constructed by use of a non-linear curve-fitting programme and the computed maximal inhibitory effects and pD_2 values are given in Table 1. The highest pD_2 value (8.7) was found for bremazocine and the lowest (7.1) for U50488; the pD_2 values of the other κ-agonists were between 8.0 and 8.3 (Table 1). The maximal inhibitory effect of the κ-agonists was between approximately 60 and 70%, with the exception of PD117302 (78.5%) (Table 1).

Table 1 Inhibitory effects of various κ-opioid receptor agonists on the electrically-evoked release of [^{3}H]-dopamine from rat striatal slices and their apparent affinities (pD_2 values) for the κ-receptors involved

κ-Agonist	*Maximal inhibition*	*pD_2 value*
Bremazocine	57.3 ± 0.3%	8.7
Ethylketocyclazocine	62.9 ± 2.6%	8.0
Tifluadom	62.9 ± 0.3%	8.3
U50488	70.1 ± 2.5%	7.1
U69593	69.3 ± 0.5%	8.0
PD117302	78.5 ± 5.4%	8.3

Data were derived from the concentration-response curves (Figure 1), computed by use of the non-linear curve-fitting programme ALLFIT and represent means ± s.e.mean of 12–16 observations. The variability in the pD_2 values was small (s.e.mean values 0.1–0.2)

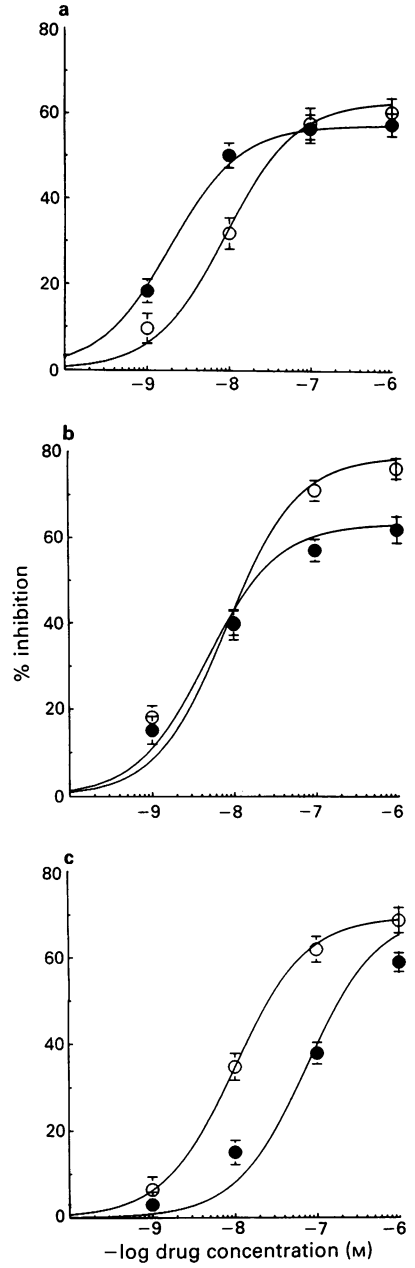

Figure 1 Inhibitory effects of various κ-opioid agonists on the electrically-evoked release of [^{3}H]-dopamine from rat striatal slices. The concentration-response curves were constructed by use of the non-linear curve fitting programme ALLFIT. The data points represent means of 12–16 observations (3–4 separate experiments); vertical bars show s.e.mean. (a) (●) bremazocine; (○) ethylketocyclazocine; (b) (●) tifluadom; (○) PD117302 and (c) (●) U50488; (○) U69593.

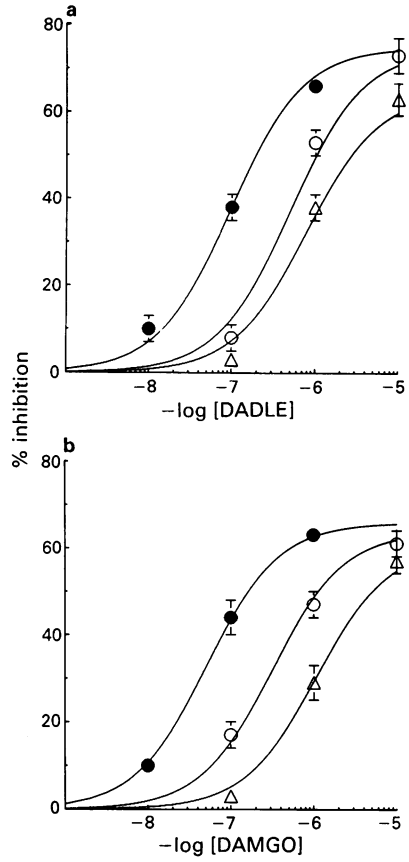

Figure 2 Competitive antagonism by bremazocine of (a) the δ-opioid receptor-mediated inhibitory effect of [D-Ala^2, D-Leu^5]enkephalin (DADLE) on (electrically-evoked) [^{14}C]-acetylcholine from striatal slices and (b) the μ-opioid receptor-mediated inhibitory effect of Tyr-D-Ala-Gly-(NMe)Phe-Gly-ol (DAMGO) on [^{3}H]-noradrenaline release from cortical slices. These experiments were carried out by use of a cumulative dose-response technique (see Methods). Data are means of 6 determinations (3 separate experiments); vertical bars show s.e.mean. (a) (●) DADLE alone; (○) DADLE + 10^{-7} M naloxone; (△) DADLE + 10^{-7} M bremazocine and (b) (●) DAMGO alone; (○) DAMGO + 10^{-8} M naloxone; (△) DAMGO + 10^{-7} M bremazocine.

Striatal [^{14}C]-acetylcholine release: δ-receptor agonism and antagonism

None of the κ-agonists significantly affected the electrically-evoked release of [^{14}C]-ACh from striatal slices, with the exception of EKC, which caused a slight inhibition (of about 25% at a concentration of 1 μM) (Table 2). This inhibitory effect of EKC was fully antagonized by 1 μM naloxone (data not shown). None of the drugs appreciably affected the spontaneous efflux of radioactivity.

Table 2 Antagonism by some κ-agonists of the δ-opioid receptor-mediated inhibitory effect of [D-Ala^2, D-Leu^5]enkephalin (DADLE) on the electrically-evoked release of [^{14}C]-acetylcholine ([^{14}C]-ACh) from rat striatal slices

	[^{14}C]-*ACh release (as % of control) in presence of:*			
κ-Agonist	κ-Agonist (1 μM)	DADLE (1 μM)	DADLE + 0.1 μM κ-agonist	DADLE + 1 μM κ-agonist
Bremazocine	89.4 ± 2.7	38.8 ± 2.4	62.3 ± 2.9**	79.3 ± 3.4**
EKC	73.7 ± 3.5†	30.3 ± 3.0	33.8 ± 2.5	48.3 ± 2.6*
Tifluadom	87.6 ± 2.9	38.0 ± 1.9	37.5 ± 2.0	44.3 ± 3.1
U50488	92.0 ± 2.5	41.3 ± 2.6	39.7 ± 3.3	40.6 ± 2.8
U69593	102.0 ± 2.7	31.8 ± 2.4	31.9 ± 3.0	30.7 ± 2.7
PD117302	95.3 ± 2.2	36.7 ± 2.5	40.9 ± 2.7	34.0 ± 2.2

Data represents means ± s.e.mean of 12–16 observations from 3–4 separate experiments.
† $P < 0.001$ (vs. control).
* $P < 0.01$ (vs. DADLE alone).
** $P < 0.001$ (vs. DADLE alone).

Table 3 Antagonism by some κ-agonists of the μ-opioid receptor-mediated inhibitory effect of Tyr-D-Ala-Gly-(NMe)Phe-Gly-ol (DAMGO) on the electrically-evoked release of [^{3}H]-noradrenaline ([^{3}H]-NA) from rat cortical slices

	[^{3}H]-NA release (as % of control) in presence of:			
κ-Agonist	κ-Agonist (1 μM)	DAMGO (1 μM)	DAMGO + 0.1 μM κ-agonist	DAMGO + 1 μM κ-agonist
Bremazocine	90.6 ± 2.6	31.2 ± 2.3	79.7 ± 2.9**	93.5 ± 3.2**
EKC	91.3 ± 2.8	27.9 ± 2.5	44.9 ± 3.1*	96.6 ± 3.5**
Tifluadom	39.6 ± 3.1†	22.9 ± 2.5	25.8 ± 2.7	27.7 ± 2.1
U50488	95.0 ± 2.6	24.0 ± 1.8	23.4 ± 2.8	25.6 ± 2.4
U69593	93.1 ± 3.0	32.6 ± 2.7	33.2 ± 3.0	40.3 ± 3.3
PD117302	94.5 ± 2.5	22.3 ± 2.6	23.1 ± 2.1	25.0 ± 2.9

Data represent means ± s.e.mean of 12–16 observations from 3–4 separate experiments. EKC = ethylketocyclazocine.
† $P < 0.001$ (vs. control).
* $P < 0.01$ (vs. DAMGO alone).
** $P < 0.001$ (vs. DAMGO alone).

DADLE (1 μM) inhibited [^{14}C]-ACh release by 60–70% and this inhibitory effect was antagonized in a concentration-dependent manner by bremazocine (0.1 and 1.0 μM). At a concentration of 1 μM EKC also partially antagonized the effect of DADLE, but the other κ-agonists did not display any antagonist action at this concentration (Table 2).

Cortical [^{3}H]-noradrenaline release; μ-receptor agonism and antagonism

None of the κ-agonists significantly affected the electrically-evoked release of [^{3}H]-NA from cortical slices, with the notable and unexpected exception of tifluadom, which strongly inhibited release ([^{3}H]-NA release 40.0 ± 2.8% of control at a concentration of 1 μM; $n = 12$). The inhibitory effect of 1 μM tifluadom was fully antagonized by naloxone ([^{3}H]-NA release 72.6 ± 4.5 and 102.2 ± 3.8% of control in the presence of, respectively, 0.01 and 0.1 μM naloxone). The spontaneous efflux of tritium was not significantly changed by tifluadom, nor by the other κ-agonists.

DAMGO (1 μM) strongly inhibited [^{3}H]-NA release (by 70–80%) and this inhibitory effect was antagonized in a concentration-dependent manner by both bremazocine and EKC (Table 3). At the highest concentration tested (1 μM) none of the other κ-agonists affected the inhibitory effect of DAMGO.

Further analysis of the δ- and μ-receptor antagonist actions of bremazocine: comparison with naloxone

In a final series of experiments the antagonist activity of bremazocine (0.1 μM) was tested against increasing concentrations (1 nM to 1 μM) of either DADLE ([^{14}C]-ACh release; δ-receptors) or DAMGO ([^{3}H]-NA release; μ-receptors), by a cumulative dose-response technique. For comparison, the antagonist activity of naloxone was also determined at concentrations of either 0.1 μM ([^{14}C]-ACh release) or 0.01 μM ([^{3}H]-NA release). The virtually parallel shifts to the right of the concentration-response curves (Figure 2) indicate that bremazocine is a competitive antagonist at both δ- and μ-opioid receptors. The pA_2 values for antagonism at the δ-receptors mediating inhibition of striatal [^{14}C]-ACh release, derived from the data of Figure 2, were 8.0 and 7.6 for bremazocine and naloxone, respectively. The pA_2 values for antagonism at the μ-receptors mediating inhibition of cortical [^{3}H]-NA release (Figure 2) were 8.2 (bremazocine) and 8.6 (naloxone).

Discussion

It has been well established that the inhibitory effects of opioids on the depolarization-induced release of [^{3}H]-dopamine and [^{14}C]-ACh from rat striatal slices are mediated exclusively by κ- and δ-receptors, respectively, whereas [^{3}H]-NA release from rat cortical slices is inhibited by opioids only if they display μ-agonist activity (Mulder *et al.*, 1984; 1989; Werling *et al.*, 1987; 1988; Schoffelmeer *et al.*, 1988). In the present study we have used these selective functional paradigms of the three major opioid receptor types in the brain to examine the potencies, relative efficacies and selectivities of a number of κ-opioid agonists, belonging to different chemical classes, with respect to these receptors.

As expected, all of the κ-agonists inhibited [^{3}H]-dopamine release, without pronounced differences in their relative efficacies, with the apparent exception of PD117302. It should be noted, however, that at the concentration which caused maximal inhibition of electrically-evoked [^{3}H]-dopamine release, PD117302 also significantly increased the spontaneous efflux of radioactivity. This almost certainly results in an overestimation of the inhibitory effect on evoked release, suggesting that in fact the maximal inhibitory effects of all κ-agonists tested fall within the range of 60–70%, i.e. under the experimental conditions used in the present study. With the exception of U50488, which had the lowest potency (pD_2 7.1), all κ-agonists showed pD_2-values of 8.0 or higher, up to 8.7 for bremazocine. A relatively low potency of U50488 compared to the benzomorphans was also demonstrated by others using peripheral tissue preparations (Hayes & Kelly, 1985; Miller *et al.*, 1986; Verlinde & DeCanter, 1988). The more recently developed κ-agonists U69593 and PD117302, as well as U50488, were the most selective ones, since they did not affect striatal [^{14}C]-ACh release or cortical [^{3}H]-NA release, nor did they possess δ- or μ-antagonist activity at concentrations up to 1 μM.

The benzomorphans, bremazocine and EKC, and the benzodiazepine analogue, tifluadom, appear to be less selective than the cyclohexyl benzeneacetamide derivatives but in different ways. Although bremazocine did not act as an agonist at the δ- and μ-opioid receptors mediating inhibition of neurotransmitter release, it appeared to be a strong antagonist at these receptors. This finding is in agreement with various studies (see Introduction), carried out with peripheral tissues or with ligand-receptor binding or neurophysiological studies in the brain. In the present study the pA_2 values of bremazocine as an antagonist at μ- and δ-receptors were estimated against DAMGO and DADLE as respective agonists. Although DADLE, in addition to being a δ-opioid receptor agonist, displays μ-agonist activity it was used in the present study as a selective agonist to activate the δ-receptors mediating inhibition of striatal [^{14}C]-ACh release, because of its relatively high potency at these receptors and the fact that μ-opioid receptor activation does not appreciably affect striatal [^{14}C]-ACh release (Schoffelmeer *et al.*, 1988; Mulder *et al.*, 1989). Compared to naloxone, the apparent affinity of bremazocine for μ-receptors was found to be about two times lower and that for δ-receptors about two times higher. The pA_2 values found for naloxone with respect to δ- (pA_2 7.6) and μ-receptors (pA_2 8.6) are in accordance with the well-known

preference of this antagonist for μ-opioid receptors (Paterson *et al.*, 1983).

EKC appeared to inhibit striatal [^{14}C]-ACh release slightly, an effect that was antagonized by naloxone and might reflect an agonistic effect on δ-receptors. Like bremazocine, EKC was found to be an antagonist at both δ- and μ-receptors. The pA_2 values of EKC were not determined, but the data suggest that EKC is equipotent with bremazocine as an antagonist at μ-receptors, but is far less effective at δ-receptors. Thus, in addition to being a potent κ-agonist, EKC appears to possess partial δ-agonist as well as potent μ-antagonist properties.

Tifluadom did not significantly affect [^{14}C]-ACh release, but it slightly counteracted the inhibitory effect of DADLE, which might indicate a slight δ-antagonist action. Surprisingly, tifluadom was found to be a rather potent agonist at the μ-opioid receptors mediating presynaptic inhibition of cortical [^{3}H]-NA release. Among the κ-agonists examined, tifluadom appears to be unique in combining agonist action at μ- and κ-opioid receptors without an appreciable effect on δ-receptors and in that sense it resembles the pharmacological profile of the opioid peptide dynorphin$_{1-13}$ (Mulder *et al.*, 1989).

In summary, in contrast to bremazocine, EKC and tifluadom, which appear to display notable differences in their pharmacological profile with respect to opioid receptors, U50488, U69593 and PD117302 are highly selective for κ-receptors. In terms of potency, efficacy and selectivity U69593 and PD117302 in particular appear to be valuable pharmacological tools for studying the physiological functions of κ-opioid receptors, although at concentrations in the micromolar range the latter drug appears to interfere with striatal dopaminergic neurotransmission in a manner not related to its κ-agonist action.

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