Opioid Ligands Having Delayed Long-Term Antagonist Activity: Potential Pharmacotherapies for Opioid Abuse

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Abstract: Buprenorphine is a partial agonist at the μ-opioid receptor with long duration of action and also exhibits delayed antagonist activity. Buprenorphine is finding increasing use as a treatment agent for opioid abuse, though its low efficacy is not well tolerated by all addicts. There is interest in developing a higher efficacy version of buprenorphine and in this mini-review some of the ligands recently discovered, that share with buprenorphine a profile of agonism followed by delayed antagonism, are discussed.

Despite the considerable advances made over recent years researchers across disciplines are still attempting to elucidate the pathways underlying many of the pharmacological and behavioural effects of the opioids. For others the search remains for elusive opioid analgesics with reduced side effect profiles compared to the medications currently in use (e.g. morphine: **1a**). New pharmacotherapies are also needed for improving treatment options for the full range of opioid addiction profiles.

Currently the most commonly utilised treatment agent for opioid abuse is methadone (**2a**). Methadone is an orally active μ-opioid receptor agonist of long duration and is used as substitution therapy for heroin abuse and dependence [1,2]. By acting on the same type of opioid receptor as morphine (μ) and having high efficacy it is possible to transfer addicts from heroin (**1b**) onto methadone. Its long duration means that it can effectively suppress withdrawal for 24 – 36 hours and therefore needs only be taken once daily. When maintained on methadone patients do not feel a euphoric effect if heroin is taken, thus reducing the intake of the illicit drug. A disadvantage in the use of methadone results from its high μ-efficacy and potency. This means methadone itself has abuse liability and a similar side effect profile to heroin, including respiratory depression that results in a substantial number of methadone-related deaths particularly in non-tolerant abusers.

At the opposite extreme of the treatment spectrum to the full μ-agonists is naltrexone (**3**), a long-lived μ-antagonist. Conceptually naltrexone has many desirable properties: it blocks the effects of subsequently administered opioids thus rendering their use pointless, and itself has no reinforcing properties and hence no abuse potential [3]. However it is this lack of any reinforcing effects that limits its use to individuals who are highly motivated [1,4]. The blocking effects can be avoided by simply waiting for the effects of the antagonist to wane or increasing the dose of opioid to swamp any antagonist present.

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Sharing properties of both agonists and antagonists are the partial agonists. Partial agonists have some level of reinforcing effects but can also attenuate the effects of a subsequently administered full agonist. They are thus acceptable to a larger proportion of the addict population than naltrexone, but with a ceiling to their μ-agonists effects are safer with regards to overdose when compared to a full agonist. Buprenorphine (**4a**) [5] is one such partial agonist that is increasingly utilised for the treatment of opioid addicts. Like the methadone congener LAAM (**2b**), buprenorphine is sufficiently long-acting that dosing can be carried out three times per week.

AGONISTS/ LONG-TERM ANTAGONISTS

It has been suggested that compounds that display agonist activity, followed by antagonist activity could provide new and interesting leads in the search for alternative pharmacotherapies for opioid abuse. In a therapeutic setting this profile can be considered very desirable as the agonist effect will provide reinforcement while the delayed antagonist effects may help protect against subsequently administered opiates. This review will focus on compounds with selectivity for the μ-opioid receptor as their potential clinical use is more apparent, though compounds acting at the other opioid receptors will also be mentioned.

BUPRENORPHINE

Buprenorphine (**4a**) stimulated much of the initial interest in this area; after the agonist actions of buprenorphine have dissipated there follows a prolonged period of antagonist activity [6]. These effects of slow onset and prolonged duration are due to slow receptor kinetics which also influence buprenorphine's agonist actions which, when established take on an irreversible, or at least antagonist resistant nature. This was demonstrated in the rat-tail pressure test where in order to antagonise buprenorphine's agonist effect a 10-fold increase in antagonist dose was required when given 30 minutes after buprenorphine compared to co-administration. The kinetics of buprenorphine in *in vitro* assays parallels *in vivo* the slow

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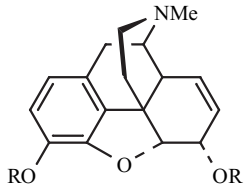
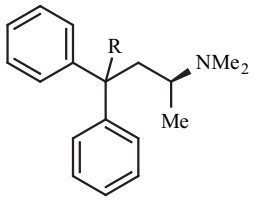
onset of its agonist actions, its long-duration of action and its resistance to antagonist reversal once the agonist activity is established. Thus in the guinea pig ileum (GPI) *in vitro* functional assays, the agonist effect of buprenorphine manifested by depression of the twitch height takes approximately 1h to develop whereas for the standard μ agonists morphine and normorphine the effect is developed within a minute or so [7,8]. Furthermore this agonist action is only slowly antagonised by even very high concentrations of naloxone and the offset from the receptor is as slow as the onset. The agonist actions of buprenorphine also display bell-shaped dose-response curves, such that once the maximum effect is reached further increases in dose only serve to reduce the agonist response. This has been demonstrated in rodent studies [6,9,10,11] and in a clinical study [12] and contribute to buprenorphine's exceptional safety profile. As yet, there is no clear explanation for this phenomenon, though both noncompetitive autoinhibition and a two receptor model have been proposed [13].

Withdrawal in addicts from high doses of morphine or heroin results in a classical abstinence syndrome that includes severe flu-like symptoms, anxiety and drug craving [14]. In contrast buprenorphine has been reported to produce few signs of abstinence and these are delayed and mild in nature [15]. Although mild, the development of an abstinence syndrome suggests buprenorphine can produce some physical dependence via its actions on the μ-opioid receptors. The low level of physical dependence produced by buprenorphine may be due in part to its low efficacy. However, other low efficacy μ-agonists, but having shorter duration of action have been shown to produce more severe abstinence syndromes suggesting that the major contributing factor is buprenorphine's slow receptor kinetics [16].

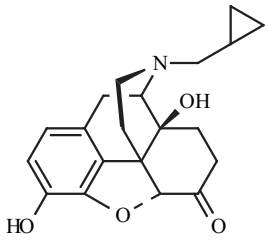
Clearly there is no mechanism by which buprenorphine can form a covalent bond with the receptor. Thus more normal receptor/ligand interactions involving the C_{20} tertiary alcohol group as well as the piperidine nitrogen,



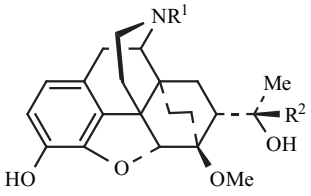
1a: R = H, morphine
1b: R = Ac, heroin



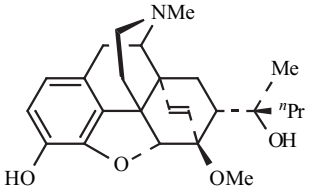
2a: R = COEt methadone
2b: R = CH(OAc)Et LAAM



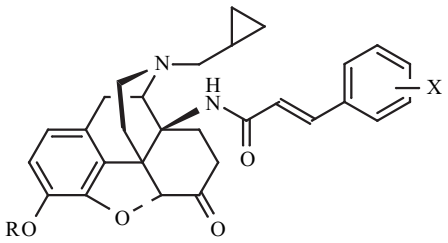
3: Naltrexone



4a: $R^1 = CH_2CH(CH_2)_2$, $R^2 = {}^t$Bu: buprenorphine
4b: R^1 = Me, $R^2 = {}^n$Pr: dihydroetorphine

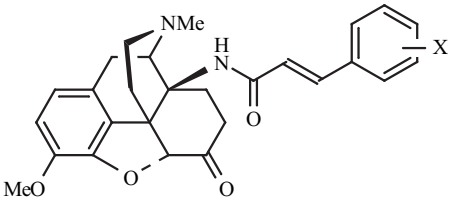


5: etorphine



6: R = H
7: R = Me

a: 4'-Cl
b: 4'-Me
c: 4'-NO_2
d: 3'-Cl
e: 3'-Me
f: 3'-NO_2
g: 2'-Cl
h: 2'-Me
i: 2'-NO_2



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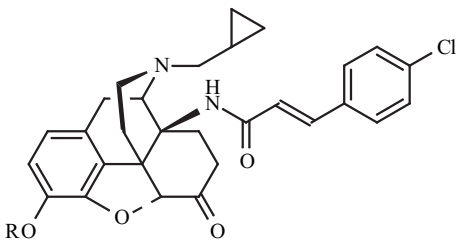


8
a: allyl
b: propargyl
c: cyanomethyl
d: propyl
e: cyclopropylmethyl
f: i-propyl
g: methoxycarbonylmethyl

cyclopropylmethyl group and the C_3-phenolic hydroxyl must account for its irreversible or long-lived character. It seems likely that the *t*-butyl group is involved in lipophilic binding to the receptor and taken together these interactions are sufficient to impart pseudo-irreversibility, preventing a subsequently administered agonist from binding, resulting in the observed antagonist activity.

The interest in buprenorphine and its' application in the treatment of drug abuse has prompted the search for other agents sharing a similar pharmacological profile. Although the low efficacy of buprenorphine is desirable in many ways, it has been suggested that a higher proportion of addicts would find buprenorphine acceptable as a treatment agent if it had somewhat higher efficacy.

14-AMINOCODEINONES AND MORPHINONES

In any review of agonist opioids having delayed antagonist actions, one series of compounds stands out as having provided a range of ligands with diverse efficacy profiles. The 14-aminocodeinones and morphinones, and in particular those having the 14-cinnamoylamino side chain, include reversible agonists, partial agonists and antagonists having no agonist actions, including those with irreversible characteristics. Of most interest to this review are the various agonists/long-term antagonists that have been found within the series. While there is still debate on the mechanism(s) resulting in the long-term effects of these opioids, the SAR data for their agonist effects is extensive.

Two of the first compounds of this structural class to be reported were the *p*-chlorocinnamoylaminomorphinone C-CAM (**6a**) and the equivalent codeinone MC-CAM (**7a**) [17-20]. C-CAM is a potent μ-antagonist with no agonist activity that displays irreversible binding characteristics and delayed onset irreversible antagonism *in vivo* allowing C-CAM to be used in the determination of relative efficacy of a range of μ-agonists [19,21]. As would be predicted from SAR generated in other series of opioid ligands (e.g. the orvinols: [22]) the methyl ether of C-CAM, MC-CAM, has higher efficacy and is in fact a μ-partial agonist *in vivo* after peripheral administration [20] with potent opioid agonist activity in the abdominal stretch assay in mice [17] and tail withdrawal assay in the monkey [20]. Interestingly MC-CAM also displays a bell-shaped dose response curve similar to buprenorphine's. This has been demonstrated in the rat-tail pressure and rat-tail flick antinociceptive assays (Lewis *et al.*, unpublished data). The anti-abdominal stretch activity of MC-CAM was not reversible by naltrexone but could be prevented by prior treatment with the antagonist, thus displaying some of the pseudo-irreversible effects typical of buprenorphine. MC-CAM has also been shown to share with buprenorphine long-lived antagonist effects. Thus, in morphine dependent, non-withdrawn rhesus monkeys MC-CAM precipitated a delayed but long-lasting withdrawal syndrome [17]. Although it represents an alternative to buprenorphine for the treatment of opioid addicts, MC-CAM's profile is not sufficiently well differentiated from that of buprenorphine's to make its further development viable. In particular it does not display the higher efficacy thought desirable in a buprenorphine alternative.

The higher efficacy of the methyl ether (MC-CAM) relative to the phenol (C-CAM) is not repeated with all ethers of C-CAM. Husbands *et al.* [23] reported on a series of 3-alkyl ethers of C-CAM (**8**) and found that while the allyl and propargyl ethers (**8a** & **8b**) had high efficacy, the cyanomethyl and propyl ethers (**8c** & **8d**) were somewhat lower and the cycloproylmethyl, isopropyl and methoxycarbonylmethyl ethers (**8e, 8f, 8g**) were antagonists in the warm water tail withdrawal (TW) assay in mice. The propargyl ether (**8b**) was studied further and shown to have activity in the tail flick (TF), hotplate (HP) and PPQ abdominal stretch assays with higher efficacy than buprenorphine or MC-CAM and as such may represent a more interesting therapeutic profile. In rhesus monkeys the propargyl ether had potency similar to MC-CAM, with both substituting for morphine in morphine dependent monkeys. Not only did the propargyl ether (**8b**) appear to have good antinociceptive activity, it also displayed delayed and prolonged antagonist effects in both the mice and monkey [23]. Thus **8b** appears to have the desired characteristics of somewhat higher efficacy than buprenorphine or MC-CAM but retaining the prolonged antagonist actions.

A significant body of data is now available relating to the effect of orientation of the substituent on the aryl ring of the cinnamoyl group of N-cyclopropylmethyl (N-CPM) codeinones (**7**). The trend is for μ-efficacy to decrease in the order *o*- > *m*- > *p*- for methyl and chloro substituents. Thus the effect of moving a methyl group from the para-position (**7b**) to the ortho-position (**7h**) is to change the profile from partial μ-agonist to very potent full μ-agonist in *in vivo* assays of antinociceptive potency after subcutaneous (s.c) administration [24]. Surprisingly, the effect of moving a nitro group from *p*- (**7c**) to *o*- (**7i**) was opposite to that just described, resulting in a reduction in agonist efficacy and potency [25]. Thus the *o*-methyl, *o*-chloro and *p*-nitro derivatives all aroused interest due to their potent agonist effects and potential for long-term μ-antagonism. The *p*-NO_2 analogue **7c** and its N-Me counterpart **9c** were both short acting agonists after i.c.v administration in the 55ºC TW assay (conditions under which MC-CAM is inactive). Pretreatment for 24 hours with either **9c** or **7c** resulted in long-term and dose-dependent antagonism of morphine antonociception [26]. However, when administered subcutaneously **7c** showed very little evidence of a delayed μ antagonist effect [25]. This disparity in results of *in vivo* antagonist studies between peripheral and central routes of administration was also shown for the N-methyl analog (**9a**) of MC-CAM. When administered *icv* **9a** had no agonist effect in TW but was a potent delayed-action morphine antagonist [26]. With peripheral administration **9a** was a potent agonist in TW, 100-times more potent than morphine with no evidence of delayed antagonism [25].

Our analogues of MC-CAM have also been evaluated in receptor binding and *in vitro* functional assays involving stimulation of [^{35}S]GTPγS binding in cloned human μ-, δ- and κ-opioid receptors transfected into chinese hamster ovary (CHO) cells [27,28]. In these assays the very substantial differences in μ efficacy between *o*- and *p*-substituted

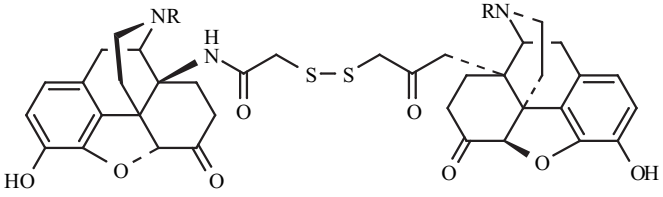
cinnamoylamino derivatives was confirmed. Thus MC-CAM (**7a**) had no μ agonist effect but was a potent μ antagonist whilst its *o*-chloro isomer was a potent partial μ agonist. In the N-methyl codeinone series the *p*-chloro analogue (**9a**) was also a μ antagonist in the GTPγS assay whereas the *o*-isomer (**9g**) was a full agonist [25]. It is interesting that the μ efficacy profiles of these compounds in the *in vitro* functional assay are in better accord with the *in vivo* profiles by *icv* administration [26] than by peripheral administration [25]. But since in a therapeutic context administration would be by peripheral routes, the data from *icv* administration may have relatively little relevance to the therapeutic potential of these compounds.

THIOL CROSS-LINKING AGENTS

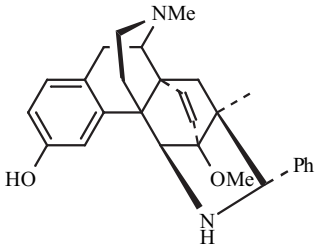
Thiol cross-linking agents alkylate receptors by forming a disulphide bond between the ligand and receptor. A number of ligands from the 14-aminomorphinone series have been designed as thiol cross-linking agents and are exemplified by TAMO (**11a**), a dithiobismorphinone [29-32]. TAMO is a μ-selective opioid with a time course of agonist and antagonist effects that make it of interest to this review. In binding TAMO displayed reasonable selectivity for μ over κ and δ, but additionally was wash resistant at μ, indicating that it could be alkylating the μ-opioid receptor by formation of a disulphide bond [31]. In the TW assay TAMO produced long acting agonism after both i.c.v and i.p administration, that was shown to be μ-mediated. Pre-treatment with TAMO resulted in delayed morphine antagonism 8-48 hours after TAMO administration [30].

BU72

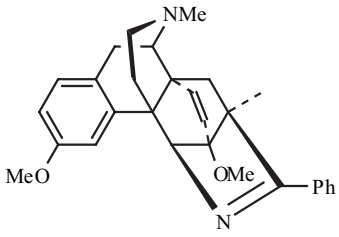
One compound that has intrigued us for some time now is the bridged morphinan BU72 (**12**). BU72 emerged from a series of ligands [33] developed from the cyclic imine (**13**) [34]. It displays non-selective, high affinity binding for all three opioid receptors [33,35]. In the GPI, MVD and GTPγS functional assay in $C_{6\mu}$ cell membranes BU72 was a potent agonist [35,36]. In antinociceptive assays in the mouse, BU72, which was non-selective in the *in vitro* assays, proved to be a selective μ-agonist with high potency (200 x methadone) and long duration of action [36,37 - designated NIH 10931]. Of particular interest from both studies was the finding of delayed μ-antagonist activity after the agonist actions of BU72 had waned. While the agonist effects at 5mg/kg lasted 8 hours, antagonist activity could not be detected until 24 hours. This was shown in both the abdominal stretch and tail-withdrawal assays. This antagonist activity was shown to be non-selective, peaking at 72 hr and lasted for 7 days at the higher doses tested



11
a: R = Me, TAMO
b: R = $CH_2CH(CH_2)_2$, NCPM-TAMO



12: BU72



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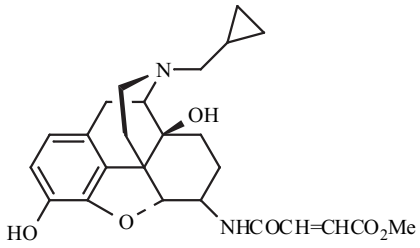
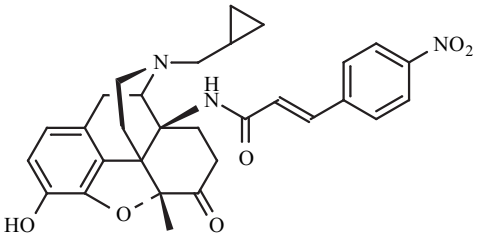


14: β-FNA



15: NCPM-MET-CAMO

(30mg/kg) after sc administration. When given by the icv route, the same pattern of activity was observed, but with an abbreviated time course. Similar results were also obtained in rhesus monkeys where a dose of BU72 (0.006 mg/kg s.c.) resulted in agonism followed by delayed antagonism that could be detected for 168 hours. Thus BU72 shares some properties with buprenorphine but is of much higher efficacy. The high efficacy of BU72 is similar to that displayed by dihydroetorphine (**4b**), an orvinol closely related to buprenorphine, but displaying very high efficacy.

DIHYDROETORPHINE

Dihydroetorphine (**4b**) and etorphine (**5**) were discovered early on in the search for opioid analgesics by Bentley and coworkers at Reckitt and Colman [38,39]. Both are extremely potent opioids (around 3000-times and 12000-times morphine respectively in measures of analgesic potency) and being highly reinforcing they have very high abuse potential. However, in the 1990's reports from China started to appear relating to dihydroetorphine (**4b**). These, and other, reports confirmed its exceptional potency, but surprisingly claimed it to have low dependence liability and to be of use in the treatment of opioid addicts [40-42]. In one study where both etorphines were evaluated concurrently, etorphine behaved similarly to dihydroetorphine [40]. Although dihydroetorphine's ability to sustain μ physical dependence is relatively low, its very high abuse potential is likely to prevent its widespread use as a treatment agent.

Dihydroetorphine has also been reported to have antagonist actions that make it of some interest to this review [43]. Depending on route of administration dihydroetorphine's antinociceptive effect in the tail flick assay at 10mg/kg peaked at 15 min (icv) or 30 min (ip) and had dissipated by 90 min (icv) and 120 min (ip). Immediately on loss of the agonist effects, the antagonist activity of dihydroetorphine could be measured. Thus after i.p administration antagonism could be detected after 2 hour pretreatment, reached a peak at 4 hours and was still significant after 6 hours. The different time course relationship of the agonist and antagonist effects between dihydroetorphine (**4b**) and the agents described previously points to a different mechanism. It has been suggested that the time course (peak antagonism immediately after the agonist effects wear off) resembles that of acute tolerance to μ-opioid receptor agonist-induced antinociception [43]. Thus the antagonist actions of the etorphines do not appear to be due to true antagonism of the receptor and are thus clearly differentiated from the ligands discussed above. A number of studies have been conducted in which cross-tolerance between various μ-opioids has been demonstrated [e.g. 49, 60-62]. It seems likely that, in this respect, etorphine and dihydroetorphine are behaving as typical μ-opioid agonists and for this reason they will not be dealt with further in this review.

Mechanisms of Biphasic Agonist/Antagonist Effects

The most readily understood mechanism of action relates to the ligands that alkylate the receptor by forming a covalent bond between the ligand and receptor. These compounds (e.g. TAMO) display an initial, reversible phase of binding followed by a second phase of binding involving formation of the covalent bond. As the pool of non-alkylated receptors dwindles, so the ligand appears more antagonist. Thus a ligand such as TAMO, which has initial agonist character will over time become antagonist. Jiang *et al.* confirmed that for TAMO these were true antagonist effects and not simply the result of cross-tolerance [30]. Unfortunately in studies of schedule-controlled behaviour and thermal antinociception in rhesus monkeys, TAMO was a long-acting μ-agonist but with no evidence of any delayed antagonist effects [32]. The agonist effects were both preventable and reversible. The lack of antagonism may reflect species differences or may simply result from the relatively low dose of TAMO that could be studied, higher doses proving toxic.

Buprenorphine, the prototype of this group of opioids cannot form a covalent bond to the μ receptor yet the kinetics of its interaction with the receptor are abnormally slow, to the extent of being pseudo-irreversible. Thus the same rationale used to explain the delayed antagonist activity of TAMO can be applied to ligands such as buprenorphine.

The 14-cinnamoylamino morphinones and codeinones appear to achieve their long-term antagonist effects by a related mechanism, though the precise details have been the subject of debate for some years. The unsaturated side chain could be acting as a Michael acceptor and alkylating a suitably placed thiol containing amino acid on the receptor, thus behaving in a manner analogous to TAMO as described above. However there does not appear to be any direct evidence for this, and in fact the available evidence would suggest that a covalent bond is in fact not formed [44,26]. Apparent confirmation of this finding appears to be provided by equivalent members of the dihydrocinnamylamino series, in which there is no possibility of covalent bond formation since they also display long-lived antagonism (Lewis *et al.*, unpublished data). Perhaps the most likely explanation is that these ligands are acting in a similar manner to buprenorphine. Thus tight, lipophilic binding leads to prolonged occupation of the receptor. Traynor and colleagues [45] have proposed that this may be the result of the cinnamoyl side chain binding not just with the receptor, but also projecting out beyond the protein and interacting with the cell membrane. All evidence so far collected certainly points to the cinnamoylamino side chain being the dominant binding motif in these series. Whether irreversible through covalent bond formation or through tight non-covalent interactions the ultimate effect of the cinnamoylamino morphinones and codeinones, and buprenorphine, is the same as for alkylating agents such as TAMO, i.e. depletion of the receptor pool.

The role of the C_3-moiety in determining the pharmacological profile of these ligands has also been studied. SAR from many series of opioids demonstrate that the 3-phenol has higher affinity than the corresponding 3-deoxy (3-H) and 3-methoxy analogues. By contrast in the cinnamoylamino series deoxy-C-CAM (DOC-CAM), MC-CAM and C-CAM all had equivalent affinities for opioid

receptors indicating that in this case the 3-position has little influence on affinity [46]. However, there is no doubt that, as in other series of opioid ligands, the 3-methoxy ligands (eg. MC-CAM) have higher efficacy than their 3-hydroxy analogues (eg. C-CAM). Metabolism studies have shown that MC-CAM is metabolised to C-CAM by O-demethylation [20]. It has been suggested that this could explain the delayed long-term antagonist effects of MC-CAM. Thus the methyl ether would provide the μ-efficacy and metabolism to the phenol would provide the irreversible antagonism. However the relationship between the effects of the codeinones administered peripherally and centrally, provides evidence that the antagonist effects of the ethers must be accounted for without the involvement of metabolism. Direct administration into the brain should minimise the effects of metabolism yet the codeinones showed much more pronounced μ antagonist effects when administered icv [26] than with s.c. administration [47]. Thus it appears that the delayed antagonist actions of the codeinones must be largely a property of the intact ligands and not due to metabolism. In contrast, 3-O-deoxygenation to DOC-CAM did affect the extent of irreversible character. DOC-CAM has a shorter duration of action that C-CAM, appearing as a reversible, yet powerful μ-antagonist [48]. Thus irreversibility appears to require both the presence of an oxygen atom at C_3 and an appropriate C_{14} side chain, such as cinnamoylamino.

The most extensive studies into the delayed, long-term antagonist actions of these ligands have been carried out in the laboratories of Bidlack and co-workers [49,26]. As already discussed this group have shown that the antagonist effects of this series were not due to cross-tolerance and were genuine antagonist effects. They have also been intrigued by the apparent dichotomy that exists between the rapid appearance of wash-resistant binding with irreversible opioid antagonists compared with the delayed onset of antagonism of opioid-agonist mediated effects, such as antinociception [49]. This appears to hold true for all irreversible opioid antagonists, not just those from the 14-cinnamoylamino series. Thus while β-FNA (**14**) requires 24 hour pre-treatment before achieving maximal antagonism of μ-opioid induced antinociception, it exhibits wash-resistant binding to μ-receptors that is complete within minutes. Similar results are obtained with irreversible antagonists of the δ-opioid receptor [50-52]. β-FNA (**14**), N-CPM-TAMO (**11b**) and N-CPM-MET-CAMO (**15**) all antagonised morphine induced analgesia with peak effects after 24 hour pre-treatment [49]. When much larger doses of the antagonists were administered the onset of antagonism of analgesia was much more rapid (≤ 140 min). The ability of the compounds to block the development of acute morphine tolerance was also measured. The results showed clearly that acute morphine tolerance was prevented at times well before the appearance of antagonism of antinociception confirming the results of binding studies which showed that the ligands are clearly interacting with the μ-opioid receptors rapidly. In later work [26] the same group suggest that the delay in antagonism of the antinociceptive effect may be due to a large receptor reserve. Thus irreversible ligand binding to a receptor causes receptor turnover and antagonist effects are only observed when a sufficient number of the total population of receptors are alkylated. Although not explicitly discussed within the paper, this may also account for the more immediate blocking of acute morphine tolerance. If irreversible binding to the receptors causes new receptors to take their place, the onset of tolerance would be delayed until a point at which no new receptors are available.

The more rapid onset of antagonist effects observed with increasing dose of antagonist and, presumably, by central as opposed to peripheral administration may simply be the result of faster receptor turnover caused by the increase in concentration of the ligand. It is also interesting to consider whether this has any relationship to the bell-shaped dose-response curve demonstrated for buprenorphine (**4a**) and MC-CAM (**7a**) in which increases in dose beyond peak effect resulted in a decrease in efficacy, i.e. an increase in antagonist character. Thus at the higher doses studied the onset of antagonism may be sufficiently rapid to reduce the level of agonism seen. It is feasible that this may contribute to the apparent lower efficacy of compounds from this series administered icv compared to peripheral administration. Thus the icv doses used may be on the descending limb of the dose-effect curve. There is clearly need for more directed research if the mechanism resulting in the bell-shaped dose-response curve is to be clarified.

In the GPI BU72 behaved as a potent agonist that could not readily be reversed by selective opioid antagonists CTAP (μ) or norBNI (κ) [35]. The wash resistant binding in GPI was intriguing as like buprenorphine, BU72 cannot form a covalent bond with the receptor. The prolonged agonist and delayed antagonist activity of BU72 *in vivo* appeared to confirm its similarity to buprenorphine and MC-CAM and suggested a similar mode of tight/lipophilic interactions with the receptor. Somewhat surprisingly it has proven possible with naloxone, albeit at high dose, to antagonise BU72's agonist actions after they have become established [36]; something that could not be achieved readily with buprenorphine or MC-CAM. These agonist effects of BU72 reappeared as the effect of the antagonist (naloxone) wore off. The antagonism displayed by BU72 was not apparent immediately after the agonist effects dissipated and they persisted for a considerable period of time, suggesting that they were not the result of simple cross-tolerance. Thus the antagonist effects of BU72 are differentiated from the cross-tolerance of the etorphines and more closely resemble the antagonist effects of buprenorphine and MC-CAM. It may be that BU72 is being localised in tissue around the receptor and while it can be displaced from the receptor by an antagonist, there is always a high concentration of BU72 present and it is able to repopulate the receptor. A similar rationale was used by Portoghese's group to account for the persistent agonist actions of κ-agonists related to naltrexone [53,54] in MVD and GPI preparations. Thus, in the GPI N-benzoyl-β-naltrexamine displays κ-agonist activity that can be reversed by naltrexone, but returns after washing the tissue. Presumably this prolonged and repeated exposure of the receptor to the agonist leads to downregulation of the receptor, i.e. a reduction in total number of receptors [55] and hence functional antagonism of subsequently administered agonists. The mechanism by which downregulation occurs is beyond the scope of this review, but is a fascinating subject in its own right [55-59].

CONCLUSIONS

Although there is little direct evidence that the profile of μ-opioid agonism followed by antagonism is therapeutically relevant to the treatment of opioid abuse, the use of buprenorphine at least supports the hypothesis. There are a number of ligands that display this pharmacological profile, several of these have efficacy higher, and in some cases considerably higher, than displayed by buprenorphine. While it is relatively easy to compare the agonist actions of the various ligands, the delayed antagonist activity is harder to quantify. There is clearly further research needed to elucidate the role, extent or even desirability, of this delayed antagonist activity in the treatment of opioid abuse.

REFERENCES

[1] Jaffe, J.H.; Martin, W.R. Opioid analgesics and antagonists. In Gilman A.G.; Rall, T.W.; Nies, A.S.; Taylor, P. (eds), The Pharmacological Basis of Therapeutics. New York, Pergamon Press, **1990**, pp 485-521.

[2] Dole, V.P. *JAMA*, **1988**, *260*, 3025-3029.

[3] Martin, W.R.; Jasinski, D.R.; Mansky, P.A. *Arch. Gen. Psychiatry*, **1973**, *28*, 286-295.

[4] Schecter, A. *Am. J. Drug Alcohol Abuse*, **1980**, *7*, 1-18.

[5] Cowan, A.; Lewis, J.W. Buprenorphine. Wiley-Liss, Inc., New York, **1995**, 326p.

[6] Cowan, A.; Lewis, J.W.; MacFarlane, I.R. *Br. J. Pharmacol.*, **1977**, *60*, 537-545.

[7] Schulz, R.; Herz, A. in *Opioids and Endogenous Opioid Peptides*, Elsevier, Amsterdam, **1976**, pp 319-326.

[8] Lewis, J.W.; Rance, M.J.; Sanger, D.J. *Advances in Substance Abuse*, **1983**, *3*, 103-154.

[9] Cowan, A.; Doxey, J.C.; Harry, E.J.R. *Br. J. Pharmacol.*, **1977**, *60*, 547-554.

[10] Rance, M.J.; Lord, J.A.H.; Robinson, T. in *Endogenous and Exogenous Opiate Agonists and Antagonists*, Way, E.L. (ed.), **1980**, Pergamon Press, New York, pp387-390.

[11] Dum, J.E.; Herz, A. *Br. J. Pharmacol.*, **1981**, *74*, 627-633.

[12] Schmidt, J.F.; Chraemmer-Jorgensen, B.; Pederson, J.E.; Risbo, A. *Anaesthesia*, **1985**, *40*, 583-586.

[13] Cowan, A. in *Buprenorphine*, Cowan, A.; Lewis, J.W. (eds), Wiley-Liss, Inc., New York, **1995**, pp31-47.

[14] Jaffe, J.H. in Gilman, A.G.; Rall, T.W.; Nies, A.S.; Taylor, P. (eds): *The Pharmacological Basis of Therapeutics.* New York: Pergamon, **1990**, pp522-573.

[15] Jasinski, D.R.; Pevnick, J.S.; Griffith, J.D. *Arch. Gen. Psychiatry*, **1978**, *35*, 501-516.

[16] Negus, S.S.; Woods, J.H. in *Buprenorphine*, Cowan, A.; Lewis, J.W. (eds), Wiley-Liss, Inc., New York, **1995**, pp31-47.

[17] Aceto, M.D.; Bowman, E.R.; May, E.L.; Harris, L.S.; Woods, J.H.; Smith, C.B.; Medzihradsky, F.; Jacobson, E.A. *Arzneim.-Forsch./Drug Res.,* **1989**, *39*, 570-575.

[18] Lewis, J.W.; Smith, C.F.C.; McCarthey, P.S.; Kobylecki, R.J.; Myers, M.; Haynes, A.S.; Lewis, C.J.; Waltham, K. *NIDA Res. Monogr.*, **1988**, *80*, 136-143.

[19] Comer, S.D.; Burke, T.F.; Lewis, J.W.; Woods, J.W. *J. Pharmacol. Exp. Ther.*, **1992**, *262*, 1051-1056.

[20] Woods, J.H.; Lewis, J.W.; Winger, G.; Butelman, E.; Broadbear, J.; Zernig, G. *NIDA Res. Monog.*, **1995**, *147*, 195-219.

[21] Zernig, G.; Butelman, E.R.; Lewis, J.W.; Walker, E.A.; Woods, J.H. *J. Pharmacol. Exp. Ther.,* **1994**, *269*, 57-65.

[22] Lewis, J.W.; Readhead, M.J. *J. Med. Chem.*, **1970**, *13*, 525.

[23] Husbands, S.M.; Sadd, J.; Broadbear, J.H.; Woods, J.H.; Martin, J.; Traynor, J.R.; Aceto, M.D.; Bowman, E.R.; Harris, L.S.; Lewis, J.W. *J. Med. Chem.*, **1998**, *41*, 3493-3498.

[24] Neiland, N.P.R.; Carrington, S.; Lewis, J.W. *Analgesia*, **1995**, *1*, 611-614.

[25] Neiland, N.P.R. Ph.D Thesis, University of Bristol, UK., **1998**.

[26] McLaughlin, J.P.; Hill, K.P.; Jiang, Q.; Sebastian, A.; Archer, S.; Bidlack, J.M. *J. Pharmacol. Exp. Ther.*, **1999**, *289*, 304-311.

[27] Traynor, J.R.; Nahorski, S.R. *Mol. Pharmacol.,* **1995,** *47,* 848-854.

[28] Toll, L.; Berzetei-Gurske, I.P.; Polgar, W.E.; Brandt, S.R.; Adapa, I.D.; Rodriguez, L.; Schwartz, R.W.; Haggart, D.; O'brien, A.; White, A.; Kennedy, J.M.; Craymer, K.; Farrington, L.; Auh, J.S. *NIDA Res. Monog. Series,* **1998,** *178*, 440-466.

[29] Bidlack, J.M.; Seyed-Mozaffari, Archer, S. *Med. Chem. Res.,* **1991**, *1*, 43-46.

[30] Jiang, Q.; Seyed-Mozaffari, A.; Sebastian, A.; Archer, S.; Bidlack, J.M. *J. Pharmacol. Exp. Ther.*, **1992**, *262*, 526-531

[31] Archer, S.; Seyed-Mozaffari, A.; Jiang, Q.; Bidlack, J.M. *J. Med. Chem.,* **1994**, *37*, 1578-1585.

[32] Gatch, M.B.; Negus, S.S.; Mello, N.K.; Archer, S.; Bidlack, J.M. *J. Pharmacol. Exp. Ther.*, **1996**, *278*, 1282-1289

[33] Husbands, S.M.; Lewis, J.W. *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 2969-2974.

[34] Lewis, J.W.; Readhead, M.J. *J. Chem. Soc. (C)*, **1971**, 1161.

[35] Husbands, S.M.; Lewis, J.W. *NIDA Res. Monogr.*, **1997**, *174*, 152.

[36] Neilan, C.L.; Husbands, S.M.; Breedon, S.; Broadbear, J.; Lewis, J.W.; Woods, J.H.; Traynor, J.R. *NIDA Res. Monog.*, **1999**, *179*, 93.

[37] Aceto, M.D.; Bowman, E.R.; Harris, L.S.; May, E.L. *NIDA Res. Monogr.*, **2000**, *180*, 346-404.

[38] Bentley, K.W.; Hardy, D.G. *J. Am. Chem. Soc.*, **1967**, *89*, 3281-3292.

[39] Bentley, K.W.; Lewis, J.W. in *Agonist and Antagonist Actions of Narcotic Drugs.* Kosterlitz, H.W.; Collier, H.O.J.; Villareal, J.E. (eds), MacMillan, London, **1972**, pp 7-16.

[40] Aceto, M.D.; Harris, L.S.; Bowman, E.R. *Eur. J. Pharmacol.*, **1997**, *338*, 215-223.

[41] Wang, S.F.; Yan, D.K.; Zang, Z.; Qin, B.Y.; Wei, Y.J.; Li, F.Y. *Chin. J. Clin. Pharmacol.*, **1992**, *8*, 106-112.

[42] Tokuyama, S.; Takahashi, M.; Kaneto, H. *Biol. Pharm. Bull.*, **1993**, *16*, 774-777.

[43] Kamei, J.; Suzuki, Y.; Nagase, H. *Neurosci. Lett.*, **1996**, *215*, 87-90.

[44] Zernig, G.; Lewis, J.W.; Woods, J.H. *Analgesia*, **1995**, *1*, 874.

[45] Traynor, J.R.; Woods, J.H.; Husbands, S.M.; Lewis, J.W. *Drug Alc. Depend.*, **2001**, *63*, S159.

[46] Derrick, I.; Neilan, C.L.; Andes, J.; Husbands, S.M.; Woods, J.H.; Traynor, J.R.; Lewis, J.W. *J. Med. Chem.*, **2000**, *43*, 3348-3350.

[47] Lewis, J.W.; Nieland, N.P.R.; Broadbear, J.H.; Woods, J.H. Presented to *College on Problems of Drug Dependence (CPDD)*, 60th Annual Scientific Meeting, **1998**.

[48] Lewis, J.W.; Derrick, I.; Neilan, C.L.; Andes, J.; Husbands, S.M.; Woods, J.H.; Traynor, J.R. *Drug Alc. Depend.*, **2000**, *60*, S126.

[49] Jiang, Q.; Seyed-Mozaffari, A.; Sebastian, A.; Archer, S.; Bidlack, J.M. *J. Pharmacol. Exp. Ther.*, **1995**, *273*, 680-688.

[50] Bowen, W.D.; Hellewell, S.B.; Kelemen, M.; Huey, R.; Stewart, D. *J. Biol. Chem.*, **1987**, *262*, 13434-13439.

[51] Calcagnetti, D.J.; Fanselow, M.S.; Helmstetter, F.J.; Bowen, W.D. *Peptides*, **1989**, *10*, 319-326.

[52] Jiang, Q.; Bowen, W.D.; Mosberg, H.I.; Rothman, R.B.; Porreca, F. *J. Pharmacol. Exp., Ther.*, **1990**, *255*, 636-641.

[53] Sayre, L.M.; Larson, D.L.; Takemori, A.E.; Portoghese, P.S. *J. Med. Chem.*, **1984**, *27*, 1325-1335.

[54] Portoghese, P.S. *J. Med. Chem.*, **2001**, *44*, 2259-2269.

[55] Tsao, P.; Cao, T.; von Zastrow, M. *Trends Pharmacol. Sci.*, **2001**, *22*, 91-96.

[56] Li, J-G.; Benovic, J.L.; Liu-Chen, L-Y. *Mol. Pharmacol.*, **2000**, *58*, 795-801.

[57] Pak, Y.; Kouvelas, A.; Scheidler, M.A.; Rasmussen, J.; O'Dowd, B.F.; George, S.R. *Mol. Pharmacol.*, **1996**, *50*, 1214-1222.

[58] Law, P-Y.; Hom, D.S.; Loh, H.H. *J. Biol. Chem.*, **1984**, *259*, 4096-4104.

[59] Bennett, D.B.; Spain, J.W., Laskowski, M.B.; Roth, B.L.; Coscia, C.J.; *J. Neurosci.*, **1985**, *5*, 3010-3015.

[60] Doverty, M.; Somogyi, A.A.; White, J.M.; Bochner, F.; Beare, C.H.; Menelaou, A.; Ling, W. *Pain*, **2001**, *93*, 155-163.

[61] Young, A.M.; Kapitsopoulos, G.; Makhay, M.M. *J. Pharmacol. Exp. Ther.*, **1991**, *257*, 795-805.

[62] Yoburn, B.C.; Lutfy, K.; Azimuddin, S.; Sierra, V. *Life Sci.*, **1990**, *46*, 343-350.