Butorphanol: Effects of a Prototypical Agonist-Antagonist Analgesic on κ-Opioid Receptors

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Abstract. The opioid analgesic, butorphanol (17-cyclobutylmethyl-3,14-dihydroxymorphinan) tartrate is a prototypical agonist-antagonist opioid analgesic agent whose potential for abuse has been the cause of litigation in the United States. With a published affinity for opioid receptors in vitro of 1:4:25 (µ:δ:κ), the relative contribution of actions at each of these receptors to the in vivo actions of the drug are an issue of active investigation. A body of evidence has been developed which indicates that a substantial selective action of butorphanol on the κ-opioid receptor mediates the development of tolerance to butorphanol and cross-tolerance to other opioid agonists; to the production of dependence upon butorphanol, particularly in the rodent; and to compensatory alterations in brain opioid receptor-effector systems. This perspective will identify the current state of understanding of the effects produced by butorphanol on brain opioid receptors, particularly on the κ-opioid receptor subtype, and on the expression of phosphotyrosyl proteins following chronic treatment with butorphanol.

Keywords: butorphanol, κ-opioid receptor, dependence, proteomics

Introduction

The opioid analgesic butorphanol (17-cyclobutylmethyl-3,14-dihydroxymorphinan) tartrate is available in the United States in an injectable form (STADOL®) and as a nasal spray (STADOL NS®). Synthesis of butorphanol was first reported in 1973 as a product of a program directed towards creation of narcotic antagonists in which potent clinical analgesic activity could be found at doses that did not elicit significant psychotomimetic effects (1). At normal therapeutic doses, butorphanol was concluded to possess analgesic activity with an abuse potential and dependence liability lower than that exhibited by morphine (2, 3). Nevertheless, butorphanol does possess rewarding properties (4) and dependence upon butorphanol has been demonstrated in experimental animals (5–8) as well as being reported to occur in man (9–11). In addition, diversion for the purpose of abuse in United States hospitals has been documented (12) as has been intravenous abuse of butorphanol and diphenhydramine (13). The introduction of STADOL NS® in 1991 was associated with increased reports of abuse and dependence (14). A highly publicized case of addiction and suicide linked to STADOL NS® focused public awareness on detrimental aspects of the actions of butorphanol (15).

The pharmacology of butorphanol is complex. A totally synthetic morphinan, butorphanol is considered to be a mixed agonist-antagonist opioid analgesic (7, 16) that has affinity for µ-, δ-, and κ-opioid receptor subtypes (17, 18). Our laboratories have progressively investigated the relative involvement of these subtypes and have demonstrated that agonist activity at the κ-opioid receptor is a significant component of the action of butorphanol in the rat, particularly with respect to dependence liability (for review, see ref. 19). Indeed, much of the data that demonstrate alterations in opioid surface receptors, receptor-effector coupling, and gene expression following butorphanol have been generated in a rodent model of physical dependence incurred as a result of continuous infusion of butorphanol or comparator drugs into a lateral cerebral ventricle (intracerebro-
ventricular, i.c.v.) (5) or the spinal cord (intrathecally, i.t.) (20).

Interest continues to evolve in understanding the physiological and pharmacological interrelationships among opioid receptors. Butorphanol, as a prototype for an agent that interacts significantly with µ-, δ-, and κ-opioid receptors, is a useful tool to assist in such studies. Because of the fundamental need to understand abuse liability and potential to develop dependence upon opioid agonists, a particular focus of attention must be the response of opioid receptors in tissues and cells to repeated or chronic exposure to agents such as butorphanol. This perspective will evaluate the current status of knowledge regarding the effects of butorphanol of the regulation and trafficking of κ-opioid receptors.

**Regulation and trafficking of KOR**

The κ-opioid receptor is a member of the G-protein-coupled receptor superfamily and appears to be the product of a single gene, KOR1, in several species, including mouse (21), rat (22, 23), guinea pig (24), and human (25). The protein product is approximately 380 amino acids in length and bears approximately 90% homology among the species from which it has been isolated (26). Possessing two consensus N-linked glycosylation sites on the extracellular N-terminus and a disulfide bonding cysteine pair linking extracellular loops two and three, the protein contains a glutamate residue at position 297 on the third extracellular loop that is postulated to be a critical “address” for highly selective binding of the κ-opioid receptor antagonist norbinaltorphimine (27). This agrees with earlier findings that the second and third extracellular loops determine high affinity binding of highly selective agonist ligands (28). The N-terminal extracellular domain had previously been identified as important for binding of the non-selective antagonist naloxone (28, 29).

The intracellular face of the receptor contains two tyrosine residues, Tyrα and Tyrβ, that are targets of phosphorylation by tyrosine kinase. Phosphorylation of these residues has varying effects on the affinity of the protein for the GTP-bound form of G-proteins and modulates κ-opioid receptor signaling efficiency (30). Another important phosphorylation site is a serine residue (Serγ) on the C-terminus. Co-expression of the serine-phosphorylating, G-protein-coupled receptor kinases, GRK3 or GRK5, with β-arrestin 2 is associated with homologous, agonist-induced desensitization of the κ-opioid receptor (31).

Despite the lack of diversity at the molecular level, the results from pharmacological and radioligand binding studies dating from the 1980s have been interpreted to discriminate among up to three κ-opioid receptor subtypes (32–34). The κ1 subtype binds arylacetamide-like agonists (U69,593, U50,488H, enadoline) and the antagonist norbinaltorphimine with high selectivity and affinity. In contrast, the κ2 subtype preferentially binds bremazocine, is U69,593-insensitive, and has an affinity for norbinaltorphimine that is 100-fold lower than the κ1 subtype (35). A third κ3 subtype, labeled selectively by naloxone benzoylhydrazone, has been proposed (32). Differential regional patterns of binding sites for κ1 and κ2 sites in the central nervous system have been well delineated (34, 36–39). Recently, Simonin et al. (40) using knockout mice in which opioid receptors had been eliminated, singly and in combination, argued that the κ2-opioid receptor actually represents a mixed population of the µ-, δ-, and κ-opioid receptors and is not a product of a distinct gene. However, the possibility remains that the residual binding of [1H]bremazocine, used to distinguish a κ2-opioid receptor, represents unique heterodimers of µ-δ or µ-κ proteins (41).

Butorphanol has been demonstrated to bind to the three principal opioid receptors, µ-, δ-, and κ-, with an affinity ratio of 1:4:25 (17) and exhibits a 3-, 10-, and 30-fold lower IC50 value for the µ-, δ-, and κ-opioid receptor, respectively, than does morphine (18). An abundance of pharmacological and receptor binding studies has led to the conclusion that butorphanol acts as a partial agonist at µ- and a pure agonist at κ-opioid receptors (see ref. 42), with preponderantly antagonist activity at the δ-opioid receptor (43). No study has shown any selectivity of butorphanol towards κ-opioid receptor subtypes. Thus, butorphanol-stimulated [35S]GTPγS binding in C6 glioma cells stably transfected to express the human κ-opioid receptor, but with a 4-fold lower efficacy than the highly κ-opioid-receptor-selective dynorphin derivative [N-methyl-Tyr1,N-α-methyl-Arg2-D-leu3]dynorphin A-(1–8)ethylamide (E2078) (44). This agonist activity can be contrasted directly with the responses to butorphanol in C6 cells expressing the rat µ-opioid receptor (45). Treatment of such cells with the selective rat µ-opioid receptor agonist DAMGO stimulated [35S]GTPγS binding by 360–390% over the control. The development of tolerance to the potent µ-opioid receptor agonist etorphine was shown by the fact that etorphine pretreatment reduced the maximal response to DAMGO to only 58% over the control. Butorphanol pretreatment was associated with little or no tolerance development, with maximal DAMGO-induced binding of 325% above the control. Similarly, as a partial µ-opioid receptor agonist, 24-h treatment with butorphanol could reduce Bmax for [3H]DAMGO binding in washed C6 cell membranes only to 50% of the control, while the full agonist etorphine reduced Bmax
by 97% from the control (45). The effects of opioid receptor binding in the whole organism are less clearly defined. In man, butorphanol demonstrated greater impairment of psychomotor performance and more negative subjective effects than did morphine, pentoza-
cine or nalbuphine, presumably due to butorphanol’s agonist activity at κ-opioid receptors (46). However, in pigeons, while the κ agonists, spiradoline and U50,488, partially substituted for butorphanol as discriminative stimuli, overall butorphanol exhibited greater μ-opioid receptor characteristics. Walsh et al. (47) compared butorphanol with the selective μ-opioid receptor agonist hydromorphone and the selective κ-opioid receptor agonist endonadoline in man and concluded that it produced predominantly μ-opioid-like pharmacodynamic effects with few κ-opioid symptoms. In drug discriminative tests in rhesus monkeys, butorphanol demonstrated agonist activity at κ-opioid receptors only after blockade of μ-opioid receptors (48).

Antinociceptive tolerance to butorphanol has been demonstrated in vivo in rats (49) and mice (8), although the degree of tolerance development may be lesser in butorphanol-treated than in morphine-treated rats. Cross tolerance was demonstrated to morphine and to the selective κ-opioid receptor agonist U50,488 (49). Butor-
phanol was also found to confer cross tolerance to the rate suppressing effects of both U50,488 and morphine in a rat self-administration paradigm (50). Thus, toler-
ance can be mediated to butorphanol through both μ- and κ-opioid receptors. More recently, Khotib et al. (51) have shown that while antinociceptive tolerance to the selective κ-opioid receptor agonist U50,488H in mice is associated with impaired U50,488H-stimulated binding of [35S]GTPγS to brain membranes, tolerance was also associated with enhanced binding stimulated by selective μ- and δ-opioid receptor agonists. McLaughlin et al. (52) similarly showed antinociceptive tolerance to U-
50,488, which was attributed to phosphorylation of serine 369 within the κ-opioid receptor by G-protein receptor kinase. It is likely that multiple mechanisms of tolerance are involved in responses to chronic admin-
istration of the mixed agonist-antagonist butorphanol.

These data confirm previous conclusions that butor-
phanol can serve as a potent agonist at the κ-opioid receptor, but that the pharmacological responses to butorphanol’s agonist actions at μ-opioid receptors often overwhelm κ-opioid receptor-mediated actions in complex biological systems. However, in the rat model, at least, dependence appears to be predominantly mediated by agonist actions of butorphanol at the κ-
opioid receptor (for review, see ref. 19). In particular, the relative role of butorphanol as an agonist at the κ-
opioid receptor, as opposed to the μ-opioid receptor stimulation produced by morphine, in the cellular and molecular adaptations produced during opioid depend-
ence, has been the focus of work in our laboratories.

Increasingly important are data that illustrate adapta-
tions of membrane receptors, intracellular signaling cascades, and gene expression following the production of dependence upon butorphanol. Alterations in κ-opioid receptors may represent the most proximal cellular adaptation to chronic exposure to butorphanol, effects that are consistent with its action as a κ-opioid receptor agonist. Work in rat brain tissue has shown multiple changes in κ-opioid receptor binding during chronic treatment with butorphanol in regimens that produce dependence upon the drug. Thus, κ-opioid receptors show desensitization to agonists in frontal cortical and striatal tissues (increased Kd for [3H]69,593 binding to 150 – 158% of the control), downregulation (decreased Bmax by 45% in frontal cortex), and supersensitivity to binding of the antagonist norbinaltorphimine (reduction in K, for [3H]U-69,593 by an order of magnitude) in the frontal cortex and spinal cord following prolonged infusion of butorphanol in the rat (7, 53). These data suggest that a conformational change in the κ-opioid receptor occurs during induction of dependence upon butorphanol. Tolerance development to chronic admin-
istration of the κ-opioid receptor agonist U-50,488H in the rat brain has been shown also (54), while chronic infusion of brentamocine, a potent κ-agonist with μ- and δ-antagonist activity, produced region-specific reductions in high affinity binding of [3H]brentamocine in some, but not all, rat brain areas (55). Interestingly, in the rat, acute i.p. injection of U-50,488H increased expression of the early-immediate gene product Fos in several brain regions, including the paraventricular hypothalamic nucleus and noradrenergic cell groups in the medulla. Tolerance developed to this action over five days of treatment. Injection of norbinaltorphimine on day 5 was unable to elicit either behavioral evidence of withdrawal or increases in Fos-immunoreactivity, suggesting that dependence did not develop to U-
50,488H, at least not through the κ-opioid receptor (56). This is in contrast to behavioral and neurochemical evidence of development of dependence upon butor-
phanol as mediated through the κ-opioid receptor (57, 58).

Further studies have been conducted on rat brain κ-
opioid receptor binding and protein levels during depen-
dence and following withdrawal from dependence upon butorphanol. Using a rat model of continuous i.c.v. infusion of an opioid agonist (butorphanol or morphine) and following naloxone-precipitated withdrawal from the ensuing dependence, Fan et al. (19) examined autoradi-
graphic binding of κ ([(3H]CI-9777), μ ([(3H]DAMGO), and δ- ([(3H]DPDPE) opioid receptors in brain tissue.
Withdrawal from dependence upon butorphanol was associated with enhanced $\kappa_1$-binding in the frontal cortex, posterior basolateral amygdaloid nucleus, dorsomedial hypothalamus, posterior paraventricular thalamic nucleus, hippocampus, ventral tegmental area, and locus coeruleus. No such changes were observed during withdrawal from morphine dependence. In direct contrast, reductions in binding of $\mu$-opioid receptors were noted with a roughly similar pattern during withdrawal from dependence upon morphine, but not butorphanol. The results indicate a qualitatively and quantitatively greater involvement of $\kappa_1$-opioid receptors than $\mu$-opioid receptors during withdrawal from dependence upon butorphanol, as opposed to morphine. Interestingly, the patterns for binding of $\delta$-opioid receptors were similar among the two groups. A further autoradiographic study (38) examined $\kappa_2$- ([3H]Cl-977), $\kappa_3$- ([3H]bremazocine in the presence of DAMGO, DPDPDE, and U-69,593), and total $\kappa$-opioid receptor binding ([3H]bremazocine in the presence of only DAMGO, and DPDPDE) in rats both while butorphanol-dependent (following 72 h of continuous i.c.v. infusion of butorphanol) and during withdrawal from such dependence (butorphanol-withdrawal; 7 h following cessation of infusion). Again, a region-specific pattern of increases in brain $\kappa$-opioid receptor binding (for both $\kappa_1$- and $\kappa_2$-subtypes) was noted during butorphanol withdrawal. However, roughly similar increases were also noted during the dependence phase. Finally, in the hippocampus alone, a reduction in $\kappa_2$-opioid receptor binding was noted during butorphanol-dependence and butorphanol-withdrawal. The results substantiate a unique participation of $\kappa$-opioid receptors in the actions of butorphanol in the rat brain during dependence upon and withdrawal from this agent. A compelling explanation for the increases in $\kappa$-opioid receptor number during chronic exposure to butorphanol is not forthcoming. However, increases in $\beta_2$-adrenoceptor number have been noted following chronic exposure to agonists (59) and chronic exposure to the $\mu$-opioid receptor agonist morphine has been associated with increases, decreases, and no change in brain $\mu$-opioid receptors (60, 61). It was suggested that the apparent increase did not represent increases in functional receptors, but may reflect uncoupling of receptors from intracellular effector systems or increases in intracellular, rather than membrane-inserted, receptor protein. A third study, examining $\kappa$-opioid receptor protein levels by immunoblotting methods, confirmed earlier results by demonstrating wide-spread, but regionally specific increases (with the exception of hippocampus and cortex, where reductions were noted) in brain $\kappa$-opioid receptor protein during withdrawal from dependence upon butorphanol, but not morphine (39).

**Butorphanol and signal transduction**

A variety of studies have documented intracellular and molecular alterations in brain tissue following chronic administration of butorphanol, as reviewed recently by Fan et al. (19). The effects of agonist-induced phosphorylation, desensitization, internalization, and downregulation of the $\kappa$-opioid receptor by agents other than butorphanol, have been most recently summarized by Liu-Chen (62). Desensitization and downregulation of the $\kappa$-opioid receptor can be observed using in vitro tissue preparations and cells that endogenously express the receptor or in which cloned receptors have been expressed. However, mixed results have been observed and important differences seem to be present between cells expressing the cloned rat versus human $\kappa$-opioid receptors (see ref. 62 for review).

Evidence is accumulating that aspects of both membrane-associated and intracellular signal transduction events are significantly modified during the development of dependence on butorphanol and while organisms are undergoing withdrawal from such dependence. Arguably, the first cellular adaptation to chronic butorphanol treatment is alteration in the number, structure, and/or function of $\kappa$-opioid receptors (7, 37, 39, 53), including uncoupling of $\kappa$-opioid receptors from...
their corresponding G proteins (63, 64). As noted above, the mechanism(s) underlying the marked increases in κ1-opioid receptors noted in rats dependent on, and in withdrawal from dependence on, butorphanol is(are) unclear. In fact, the increase in numbers may reflect κ1-opioid receptors that are not, or have not yet been, inserted into the plasma membrane as active receptors (39). Our laboratory has suggested that these receptors may represent a subgroup of receptors that are stored in/transported by protein secretory pathways and are not inserted into presynaptic membranes until prompted by increased activity of the neurons in which the receptors are located. The presynaptic localization of such κ1-opioid receptors, and their enhanced numbers, is consistent with a significant body of work focusing on neuronal hyperexcitability within the locus coeruleus during withdrawal from opioid dependence. In a rodent model of withdrawal from dependence on butorphanol dependence, increased extracellular concentrations of the excitatory neurotransmitter glutamate (and in some case, aspartate) are found (65), an effect that seems specific to the κ-opioid receptor (57). Moreover, the increase in concentrations of postsynaptic NMDA receptors within the locus coeruleus under such conditions (66–68) would further enhance neuronal excitability. In addition, dependence on butorphanol is associated with the development of supersensitivity of opioid receptors to binding of a narcotic antagonist (7, 53). The mechanism(s) by which this occurs are similarly not yet defined, but the end result is an enhanced ability of narcotic antagonists to elicit withdrawal behaviors (69). The association(s) of κ-opioid receptors to intracellular signal transduction elements/events, including regulation of GTPase activity (63, 64), modulations of adenyl cyclase activity (67), modifications to voltage-gated membrane potassium and calcium channels (70–72), protein kinases, as well as transcription factors, and the associated alterations in gene expression (19, 73) have all been linked to butorphanol dependence and withdrawal. These are all candidates to explain the long-lasting (67) molecular and behavioral plasticity (19, 66) associated with butorphanol treatment. A model synapse that presents the hypothesized sequence of events that occur within the locus coeruleus during the control situation, following acute administration of butorphanol, and during butorphanol dependence and acutely-precipitated withdrawal from such dependence is shown as Fig. 1. This model, and the data that support it, pose additional questions. To answer these questions, a novel analysis of the responses evoked in rat brain during development of dependence upon butorphanol has been performed by Kim et al. (74). That study is the first to utilize proteomic methodologies to identify phosphotyrosyl protein expression in the rat model of butorphanol dependence. Two-dimensional electrophoresis was used to separate phosphotyrosyl (p-Tyr) proteins, whose identity was subsequently determined using MALDI-TOF mass spectroscopy. Of 65 protein spots that could be found in frontal cortex tissue from both control and butorphanol-dependent rats, a majority were found to have a greater staining density in the butorphanol group. In addition, 18 p-Tyr spots were found uniquely in butorphanol-dependent tissue. Fifty-three of the p-Tyr spots could be identified as proteins involved in intermediary metabolism and cell signaling, although no receptor proteins were defined. In addition, proteins involved in glial and neuronal morphology and in axonal transport were detected, suggesting that chronic butorphanol treatment can alter neuron and glial structure.

Conclusion

Although potentially compromised by recent controversy in the United States, butorphanol remains a clinically useful analgesic agent. In the laboratory, butorphanol also retains significant value as a prototypical agonist-antagonist opioid receptor ligand. Resolution of the relative contributions of action at each of the primary opioid receptor subtypes in specific pharmacological responses continues to be a focus of ongoing study. However, while the results of psychomotor and drug discrimination studies have yielded mixed conclusions as to opioid receptor subtype involvement, an abundance of data indicates that agonist effects of butorphanol at κ-opioid receptors contribute to the development of tolerance and cross-tolerance; to the production of dependence upon butorphanol, particularly in the rodent; and to compensatory alterations in brain opioid receptor-effector systems.

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