In-vivo studies on kappa opioid receptors

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The actions of opioids are mediated by multiple types of opioid receptor. As a result, 'obtaining the right balance' is the catchphrase most frequently heard these days in analgesic research laboratories throughout the pharmaceutical industry. Tomorrow's analgesics will feature a prominent κ *component, a touch of* δ*, a tickle of* μ*, but not even a wisp of* σ*. New compounds are fashioned largely from structure–activity relationships involving bioassays and radioligand receptor binding. These* in-vitro *approaches have become well established over the past decade since they help to link receptor type to the analgesic under investigation. What about the complementary preclinical tests* in-vivo*? Specifically, how can the animal pharmacologist assist in characterizing* κ *opioid activity? In this article,* Alan Cowan *and* Debra E. Gmerek *present a survey of tests that are being used to detect and define* κ *activity* in-vivo*. Special emphasis is placed on the rat bombesin-scratch test, a new procedure in which several* κ*-preferring agents are selectively active.*

Compounds that are classified as κ-agonists (e.g. tifluadom, U-50488; U-50488 is *trans*3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl 1]-benzeneacetamide); usually on the basis of bioassay and binding activity profile, are generally perceived to be safer than the traditional morphine-like or μ-agonists. This view derives from the different pharmacologies of μ- and κ-agonists, a topic that has been analysed at length[1]. In short, κ-agonists are of interest because they offer diversity in chemical structure, antinociception, a milder form of physical dependence, and limited actions on respiration and gastrointestinal transit. Unfortunately, with many of the older κ-agonists, the price paid for the pleasing profile was the emergence of unwanted dysphoric and psychotomimetic (i.e. σ) side-effects. Nalorphine, the historic narcotic antagonist analgesic, was clinically abandoned for this reason. Dysphoria and altered perception have been associated with some of the more recently described κ-agonists (e.g. the benzomorphans, ketazocine and MR 2034, i.e. (−)-(1R,5R,9R,2″S), 5,9-dimethyl-2-tetrahydrofurfuryl-2′-hydroxy-6,7-benzomorphan).

The finding that ketazocine can cause unpleasant symptoms in humans[2] is conceptually important, given that this compound was introduced as the prototype agonist at κ receptors[1]. Cross-reaction between κ and σ opioid receptors is clearly a major worry in the commercial development of κ-agonists as potential analgesics. Another concern is the possibility that κ receptors *per se* (also) mediate some of the disagreeable subjective effects associated with acute administration of certain κ-agonists to humans.

A key objective of analgesic drug development throughout κ-research has been to manoeuvre between and beyond the twin threats of physical dependence and negative central effects. Many groups accepted the challenge, believing that the ideal κ analgesic, although elusive, can ultimately be designed. At the moment, a clinical opinion on full, selective κ-agonists of the tifluadom and U-50488 type has still to be formed.

Pharmacological profiles of κ-agonists in rodents

A screening cascade for κ activity conventionally starts with bioassays and radioligand receptor binding. Next, the subtle twists of antinociceptive testing are tackled. The selection of particular tests is dependent on the philosophy behind the analgesic project e.g. the relative balance of receptor activities that is perceived to be ideal for the target pain state in man. Selection of antinociceptive method and route of administration is also dependent on whether the primary focus of action is deemed to be spinal, supraspinal, or in the periphery. A pharmaceutical company searching for its own tifluadom or U-50488 might well choose mouse abdominal constriction and hot plate tests to establish levels of antinociception and reversibility by naloxone[3]. Differences between test compounds may be emphasized by using supraspinal (i.c.v.) and spinal (intrathecal) routes of administration[4–6]. For example, U-50488, ketazocine and morphine are all active after intrathecal administration in the mouse (0.6% acetic acid) writhing test but only morphine is active by the i.c.v. route. It seems that κ-receptors modulate visceral pain at spinal, but not at supraspinal, levels in this procedure (Porreca, Mosberg, Burks and Cowan, unpublished data). Note, however, that the choice of noxious stimulus is critical. Indeed, the quality of the stimulus used has long been considered a key variable in the evaluation of novel analgesics. This point is obvious when results from the mouse hot plate (55°C) test are compared. In this test, U-50488 has unimpressive efficacy and potency when given by i.c.v. or intrathecal administration whereas morphine is still active by either route[5].

The next level of testing includes procedures that help to further distinguish tifluadom and U-50488 from μ-agonists such as morphine. Several approaches are outlined in Table I. There is no common consensus on the best mix of methods. The flurothyl test[7] contrasts markedly with the bombesin-scratch test in that ethylketazocine, tifluadom and U-50488 are active in the latter and inactive in the former. Morphine is not active against bombesin but raises the seizure threshold (along with several other μ-agonists) in rats exposed to flurothyl, a volatile convulsant.

The rat bombesin-scratch test

The bombesin-scratch test (Ref. 8; see Fig. 1) is a recent addition to those *in-vivo* methods that can be used in the evaluation of compounds with activity at κ receptors. Bombesin, a tetradecapeptide originally isolated from frog skin in 1971, is one of a surprisingly large number of well-known endogenous substances which cause

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Fig. 1. Scratching caused by bombesin 0.10 μg, i.c.v.

excessive scratching and/or grooming when given centrally to rodents (e.g. prolactin, SP and vasopressin). Reasons for these behaviours have yet to be established. Neuropeptide-induced grooming/scratching in rats can be altered in different ways by subcutaneously administered morphine and naloxone[9–11]. Thus, the excessive grooming and/or scratching associated with $ACTH_{1-24}$ is attenuated by both morphine and naloxone; that associated with thyrotropin releasing hormone (TRH) is suppressed by morphine but not by naloxone; while that associated with bombesin is unaffected by both morphine and naloxone. The resistance of bombesin towards morphine prompted testing of many opioids and opioid peptides in an attempt to suppress the robust scratching elicited by bombesin, in rats. The initial results revealed that several mixed agonist–antagonists/κ-agonists attenuated bombesin-induced scratching in a stereoselective and dose-related manner. Examples of these agents (all benzomorphans) are bremazocine, cyclazocine, ethylketazocine, ketazocine and (−)pentazocine; they seem to share pharmacological effects in rats that dispel the animals' preoccupation with scratching. Other commonly used opioids (mainly μ-agonists) and opioid peptides were ineffective against bombesin when tested at behaviourally nondepressant doses. Examples of inactive compounds were buprenorphine, levorphanol, meperidine, methadone, metkephamid and β-endorphin. The scratching was essentially unaffected by behaviourally nondepressant doses of haloperidol, indometacin, lidocaine, neurotensin and several antihistaminic/antiserotoninergic agents.

Suppression of bombesin-induced scratching by ethylketazocine seems to involve stereospecific opioid binding sites since (−) naloxone, but not (+)naloxone, attenuated the antibombesin effect of ethylketazocine[8]. The following observations implicate κ, rather than μ, binding sites: (1) multiple injections of morphine did not influence the ability of ethylketazocine to antagonize bombesin, and (2) when μ-receptors were blocked with buprenorphine, ethylketazocine still antagonized bombesin-induced scratching.

One puzzling result was the inclusion of phenazocine (generally regarded as a μ agonist *in vivo*) in the list of active agents (Table II). Since all compounds showing antibombesin effects were benzomorphans (including phenazocine), the question of κ- v. benzomorphan-selective binding sites needed to be addressed. Recent experiments with nonbenzomorphan κ-agonists (tifluadom and U-50488H) showed that these agents are active in the procedure (Table II) and can be antagonized in a dose-related manner by naloxone. It may be concluded that the bombesin-scratch test is a useful addition to the list of κ evaluative methods outlined in Table I.

As a postscript to the test, note that there is a link between this less common measure – scratching – and κ compounds, that stretches back to the early 1970s. At that time, it was noticed that monkeys, receiving κ-directed mixed agonist–antagonists such as cyclazocine and nalorphine for a month, scratched excessively when they were withdrawn from these agents or were challenged with naloxone[12]. Scratching was also observed during withdrawal from certain bridged oripavines, compounds that were classified as κ-preferring almost a decade later[13]. Recent work from the University of Michigan has shown that rhesus monkeys undergoing abrupt withdrawal from U-50488 (but not from morphine) also display an unusually high incidence of scratching[14]. Interestingly, this behaviour is suppressed by tifluadom but not by morphine.

Other tests

Other tests show that morphine-like agonists have antidiuretic effects. κ-Agonists are completely

TABLE I. Pharmacological profiles of test agents after peripheral administration

Procedure	Ethylketazocine	Tifluadom	U-50488	Morphine
Rats				
Bombesin-scratch test	+	+	+	−
Increased flurothyl thresholds	−	−	−	+
Diuresis	+	+	+	−
Increased food intake	+	+	+	±
Inhibition of gastrointestinal transit	+	−	−	+
Rhesus monkeys				
Nalorphine discrimination	+	+	+	−
Morphine-withdrawal suppression	−	−	−	+

+ = positive or dose-related effect. ± = equivocal. − = no consistent effect.

TABLE II. Effects of representative opioids and opioid peptides on bombesin-induced scratching in rats[a]

Test agent	A_{50} (mg kg^{-1} s.c.)
Ethylketazocine	0.36 (0.33–0.40)[b]
U-50488	6.61 (5.37–8.13)
Tifluadom	6.80 (5.52–8.36)
Phenazocine	0.29 (0.19–0.43)
Morphine	>10[c]
Meperidine	>25
Metkephamid	>30
β-endorphin	>10 μg i.c.v.

[a]The standard dose of bombesin was 0.10 μg i.c.v. [b]95% Confidence limits. [c]Highest behaviourally nondepressant dose tested.

different. Water diuresis seems to be an inherent property of virtually all known κ-agonists, at least in rats and monkeys (ketazocine and MR 2033 are diuretic in man; MR 2033 is (±)-(1R,5R,9R,2″s)-5,9-dimethyl-2-tetrahydrofurfuryl-2′-hydroxy-6,7benzomorphan). One current theory is that κ-agonists suppress vasopressin release from the neurohypophysis and this leads to increased urine output. There may be a peripheral site of action in the rat since bilateral adrenal demedullation abolishes κ-mediated diuresis[15]. At a practical level, diuresis is undoubtedly the most popular approach *in vivo* for comparing new and standard κ directed ligands. Reasons for this are quite clear. Increased urine output represents a simple, distinguishing feature of κ-agonists. Examination of dose–response curves, along with interactional studies with morphine, bremazocine and β-funaltrexamine (i.e. the β-fumaramate methyl ester of naltrexone) (an irreversible μ-antagonist *in vitro*), allows differentiation within the κ class e.g. ethylketazocine from U-50488 and from nalorphine[16]. Parenthetically, κ-preferring opioid peptides also seem to be active in the diuresis test since dynorphin-A, the endogenous κ ligand, increases urination within 1–2 h of i.c.v. administration to normally hydrated rats[17]. [D-Pen^2, D-Pen^5]enkephalin, probably the most selective agonist at δ-opioid receptors that is currently available[18], has no marked influence on urine output[17].

Dynorphin-A (i.c.v.) initiates feeding in rats. This action is antagonized by naloxone. Ethylketazocine, tifluadom and U-50488 likewise induce feeding (after s.c. injection) but morphine is often ineffective in drug-naive rats[19]. Morphine can be more clearly distinguished from tifluadom and U-50488 in the rat charcoal meal test. In this procedure, morphine delays the passage of charcoal along the GI tract in a dose-related manner but tifluadom and U-50488 are without major effects[20]. Ethylketazocine resembles morphine rather than tifluadom and U-50488 in this test; the antitransit action of ethylketazocine is probably mediated by μ-receptors.

Studies with κ-agonists in monkeys

Knowledge that several opioids can function as discriminative stimuli to control the behaviour of animals is utilized in more advanced levels of testing. For the evaluation of κ activity, drug discrimination experiments use animals that are trained to respond in either one way or another depending on the presence or absence of the interoceptive cues associated with injection of κ-agonists. The work of Tang and Code[21] provides a good example. They found that rhesus monkeys, trained to discriminate the pharmacological effects of nalorphine (a κ-agonist in this species) from saline, generalized completely to ethylketazocine, tifluadom and U-50488 but (critically) not to three μ-agonists – morphine, methadone and meperidine.

Monkeys have also been used in morphine-withdrawal suppression studies (at the University of Michigan) to characterize κ-directed ligands. Behaviourally nondepressant doses of ethylketazocine, tifluadom and U-50488 (in contrast to morphine) do not suppress signs of withdrawal in 14 h withdrawn, morphine-dependent monkeys[14]. In these animals, a well-known behavioural syndrome emerges (e.g. excessive vocalization, abdominal defense reactions, vomiting)[22]. The syndrome associated with deprivation-induced withdrawal from U-50488 is qualitatively different; as already indicated, scratching is prominent, along with picking at fingers and toes, and frequent grooming of other monkeys[14].

Future trends

Discovering new and better analgesics is no easy matter. We may have to settle for less than ideal opioids, and only modest gains, at least in the short term. Perhaps the growing emphasis on 'nonopioid' pain modulating systems, both central and peripheral, will foster a rate of drug introduction more in keeping with academic advances. In the meantime, opioids with various activities at μ, κ and δ receptors are undergoing clinical trials. The evaluation of key compounds from chemical classes as disparate as benzeneacetamide (U-62066; 5α,7α,8β-(±)-3,4-dichloro-*N*)-methyl-*N*(7-(1-pyrrolidinyl)-1-oxaspiro-(4,5)dec-8-yl) benzene + acetamide) and morphinan (xorphanol) should provide the missing and vital links between clinical analgesia, subjective effects, antinociception and balance of receptor activities. For better or for worse, results from studies such as these will have a profound influence on the way analgesic researchers pursue elusive anodyne.

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Books

Short and concise handling of drug interactions

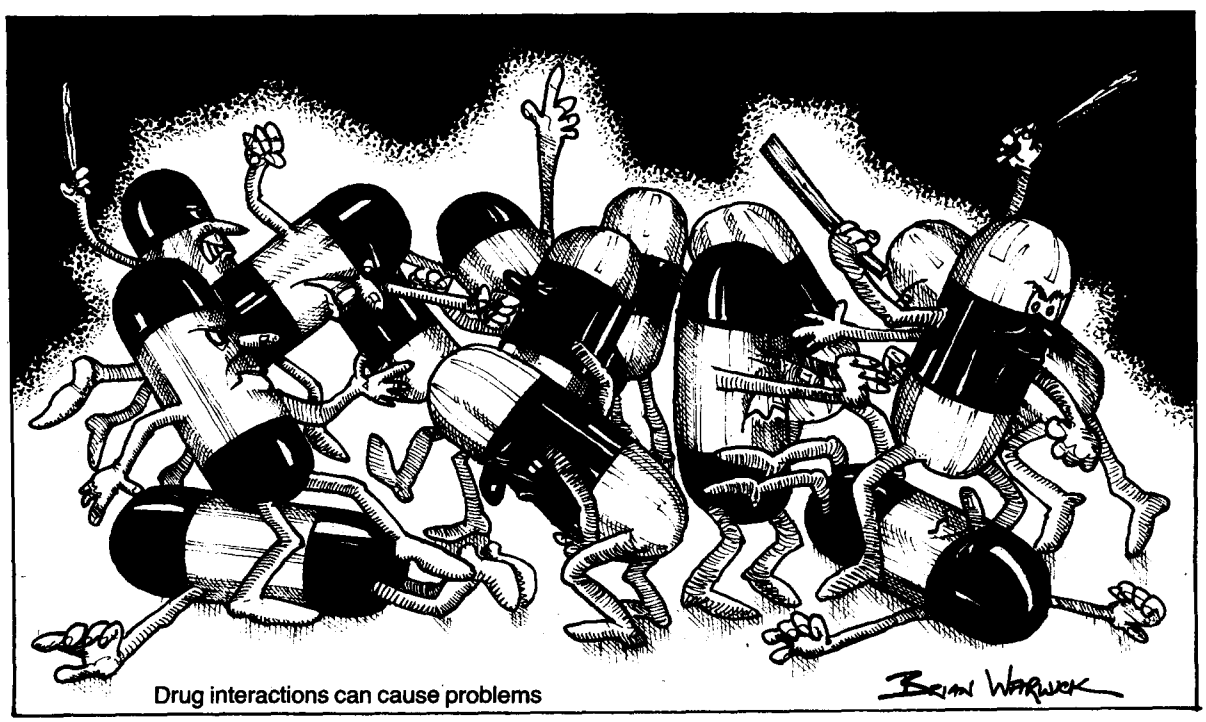
Drug interactions, 5th edn

by Philip D. Hansten, Lea & Febiger, 1985. $24.75 (x + 460 pages) ISBN 0 8121 0944 9

There are so many drugs available that it is quite impossible for anyone to be able to remember with any certainty more than a handful of drugs which can, and which cannot, be safely administered together. The philosophy behind the writing of the first edition of this book, published in 1971, and behind the writing of all of the subsequent editions, has been to offer a solution to the problem of drug interactions by providing concise information in a helpful and down-to-earth style which can be used when drugs are being prescribed or dispensed. So it is essentially a practical reference book. If you want a discursive and detailed treatise on the pharmacological mechanisms which underly the interactions between drugs, then this book is not for you (although you might find it very helpful in pointing your nose in the right direction). But if, on the other hand, you are looking for a practical manual which offers direct guidance, then this book will fit the bill very nicely.

The author has divided the book into a number of chapters which neatly categorize drugs into therapeutic or chemical groupings ('Anticoagulants', 'Anticonvulsants', 'Phenothiazines', etc.) and then subdivided the drugs which fall into these categories alphabetically. A further subdivision allows the interactions of particular drug–drug pairs to be dealt with in summary form under the headings of 'Mechanism', 'Clinical Significance' and 'Management'. The summaries are for the most part short and concise, and are referenced with superscript numbers which refer to bibliographies at the end of each chapter. Generally speaking, the size of the summary is some indication of the extent of the knowledge of the interaction in question. And, of course, as is to be expected of a reference book, there is a good index at the back of the book which uses for the most part generic names, but with a sprinkling of proprietary names as well. Those familiar with previous editions will note that the section which was given over to effects on clinical laboratory results is missing. This, we are told in the introduction, is to be published separately later.

Professor Hansten is one of the



Drug interactions can cause problems