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Differential sensitivity of antinociceptive tests to opioid agonists and partial agonists

[1]John S. Shaw, Janet D. Rourke & Karen M. Burns

Bioscience Department, ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG

1 The antinociceptive activity of a range of opioid agonists and agonist-antagonist analgesics was determined in mice by use of the 55°C hot plate and abdominal constriction assays.

2 Opioid agonists were approximately 10 times more effective in the abdominal constriction assay.

3 The agonist-antagonists produced analgesia only in the abdominal constriction assay, and antagonized the antinociceptive action of opioid agonists in the 55°C hot plate test.

4 These differences were shown to be attributable to the different levels of stimulus employed in the two tests.

5 By comparing the antagonist potencies of the agonist-antagonists in the 55°C hot plate test with their antinociceptive ED_{50} values in the abdominal constriction assay, an index of intrinsic activity was calculated.

Introduction

A variety of methods have been developed to assess analgesic activity in rodents. Whilst each of these techniques is capable of detecting drugs with properties similar to those of morphine, they vary considerably in their sensitivity (Tyers, 1980; Ward & Takemori, 1983). Moreover, some commonly-used analgesic tests, especially those employing heat as the noxious stimulus, do not reliably detect analgesics of the opioid 'agonist-antagonist' (Martin, 1967) class. For example, drugs such as nalbuphine, buprenorphine and pentazocine are reported to have little effect on reaction time in response to thermal stimuli (O'Callaghan & Holtzman, 1975; Sewell & Spencer, 1976; Luttinger, 1985; Zimet *et al.*, 1986). However, these drugs produce potent antinociceptive effects against a wide range of chemical stimuli in abdominal constriction models (Taber *et al.*, 1964; Collier *et al.*, 1968; Tyers, 1980).

In an attempt to explain these observations, Tyers (1980) proposed that the transmission of heat and non-heat nociceptive information may involve different neuronal pathways which possess different classes of opioid receptors. Thus the heat pathway would involve μ- but not κ-receptors and would thus be sensitive to morphine-like agents, but not to the agonist-antagonist analgesics which act predominantly at κ-sites. The non-heat pathway would involve both μ- and κ-receptors. Whilst this theory explained much of the data available at the time, recent findings suggest that the hypothesis should be re-examined. In particular, two of the drugs which are ineffective in heat models of nociception, nalbuphine and buprenorphine, have been shown to act predominantly at the μ-receptor (Leander, 1983a; Miller *et al.*, 1986; Hayes *et al.*, 1985).

In the present study the potencies of a number of opioids were compared in models employing heat and chemical stimuli: the mouse 55°C hot plate (subsequently referred to as the 'Hot plate test') and abdominal constriction assays. Since it has been reported that many of the agonist-antagonist analgesics are ineffective in the hot plate model, a number of compounds which failed to produce an antinociceptive response in this assay were tested for their ability to antagonize the actions of the μ- and κ-agonists morphine and ethylketocyclazocine (EKC). By this means it was possible to determine whether these drugs merely lack affinity for the receptors which mediate the hot plate response, or whether an alternative explanation needs to be sought to explain their lack of activity.

Finally, an attempt was made to determine the extent to which the intensity of the noxious stimulus is a factor in deciding the sensitivity of an analgesic test. The antinociceptive potencies of a range of drugs were determined in the abdominal constriction model with three different levels of stimulus, i.e. concentrations of acetic acid. The results from this and

[1] Author for correspondence.

the other parts of the study indicate that the analgesic response is determined by both the intensity of the stimulus and the intrinsic activity of the analgesic.

Methods

The animals used throughout this study were female mice of the Alderley Park strain weighing between 20 and 25 g. All drugs were dissolved in physiological saline (with the addition of one equivalent of 0.5 N HCl where necessary) and were administered by the subcutaneous route 30 min before testing.

Hot plate assay

Mice were placed on a copper surface which was thermostatically maintained at 55 ± 0.2°C. The time taken to react by lifting a hind paw was recorded. Animals not reacting within 20 s were removed from the plate and assigned a score of 20 s. Results were transformed as:–

$$\% \text{ effect} = \frac{\text{response} - \text{control}}{20 - \text{control}} \times 100$$

Abdominal constriction assay

Mice were injected intraperitoneally with 0.4 ml dilute acetic acid (0.25%, 0.4% or 0.6%) and were placed in perspex observation chambers. The number of constriction responses occurring in the period from 2 to 17 min after the acetic acid was recorded. Results were expressed as percentage inhibition of the control (saline-pretreated) group.

Statistics

ED_{50} values and 95% confidence limits were calculated by least squares linear regression analysis after first checking for deviations from linearity. Antagonist potencies were calculated by Schild analysis and were expressed as AD_2 doses (equivalent to the *in vivo* apparent K_e value). The slope of the Schild plot was taken to be unity after checking that it did not deviate significantly from this value.

Drugs

The following drugs were generous gifts from the companies indicated: cyclazocine, ethylketocyclazocine, pentazocine (Sterling-Winthrop): buprenorphine (Reckitt and Coleman): bremazocine (Sandoz): nalorphine (Wellcome): nalbuphine (DuPont): xorphanol (H.G. Pars): levallorphan (Roche): fentanyl (Janssen). Naloxone and naltrexone were purchased from Sigma, and morphine and methadone from Macfarlan-Smith. The remaining drugs were synthesized within the Chemistry Department, ICI Pharmaceuticals Division.

Results

Antinociceptive potency

The potency of the prototypic opioid μ-receptor agonist morphine differed substantially between the tests (Figure 1a). Morphine proved to be approximately ten times more potent in the abdominal constriction model than in the hot plate assay. A similar pattern was observed with the κ-agonist EKC (Figure 1b).

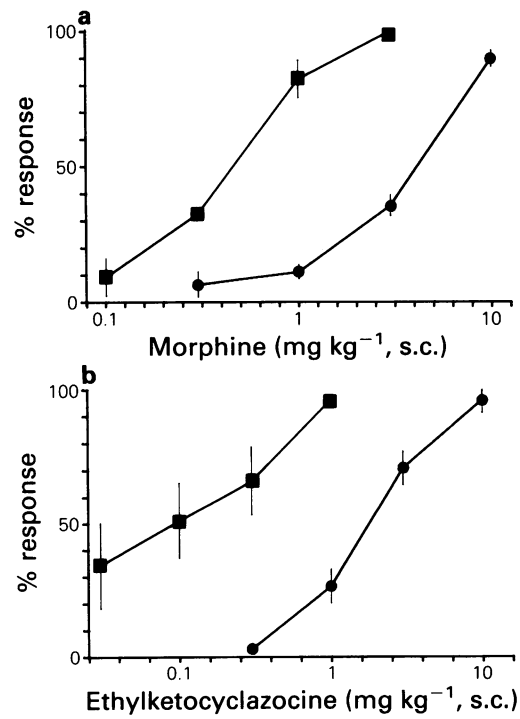

Figure 1 Dose-response curves to (a) morphine and (b) ethylketocyclazocine in the 55°C hot plate (●) and 0.4% acetic acid abdominal constriction (■) assays. Each point represents the mean of 10–20 mice; s.e.mean indicated by vertical lines.

Table 1 Potencies of a series of opioids in the mouse 55°C hot plate and 0.4% acetic acid abdominal constriction assays

Drug	*Hot plate* ED_{50}	*Abdominal constriction* ED_{50}
Morphine	3.4 (3.3–3.9)	0.41 (0.28–0.62)
Methadone	3.07 (2.5–3.8)	0.27 (0.12–0.58)
Fentanyl	0.04 (0.03–0.07)	0.032 (0.02–0.052)
EKC	1.6 (1.4–1.9)	0.17 (0.12–0.23)
Tifluadom	3.4 (2.9–3.9)	0.3 (0.23–0.45)
U50488	~30*	1.1 (0.63–2.2)
Bremazocine	>30	0.01 (0.003–0.07)
Nalbuphine	>30	1.4 (0.58–1.39)
Nalorphine	>100	0.77 (0.09–3.4)
Levallorphan	>30	1.1 (0.34–7.1)
Cyclazocine	>10	0.23 (0.11–0.57)
Xorphanol	>30	0.08 (0.04–0.19)
Pentazocine	>30	2.5 (1.1–9.1)
Buprenorphine	>10*	0.04 (0.013–0.08)
Naloxone	NA 10	NA 10
Naltrexone	NA 10	NA 10

* Shallow dose-response
NA: not active up to the dose shown.
All values are ED_{50} doses (mg kg^{-1}, s.c.) with 95% confidence limits.
EKC = ethylketocyclazocine.

Examination of a wider range of opioid analgesics (Table 1) revealed that the μ-receptor agonists, morphine, methadone and fentanyl, were all effective in both the abdominal constriction and the hot plate assays. Each of these compounds, however, was approximately ten times more potent in the abdominal constriction test. In contrast, the so-called 'agonist-antagonist' group of analgesics (bremazocine, buprenorphine, pentazocine, xorphanol, cyclazocine, levallorphan, nalorphine and nalbuphine) were effective only in the abdominal constriction model. Amongst the κ-agonists the picture was somewhat less clear. Tifluadom and EKC behaved in a similar way to morphine, being ten fold more potent in the abdominal constriction model than in the hot plate test, whilst U-50488H (*trans*-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide) although active in the hot plate test, produced a shallower dose-response curve with a reduced maximum.

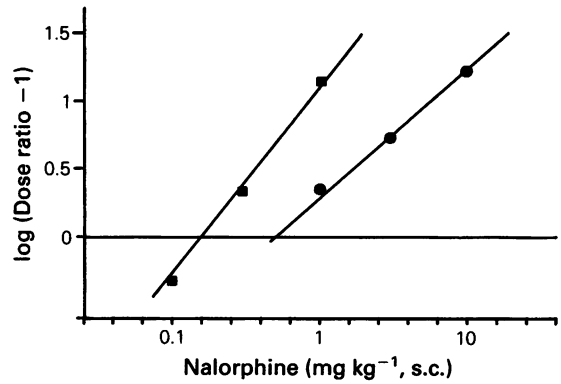

Figure 2 Schild plot for the antagonism of morphine (■) and ethylketocyclazocine (●) by nalorphine in the 55°C hot plate assay. Each point represents the mean result from 10 mice.

Antagonist activity

To investigate whether the inactivity of the agonist-antagonist compounds in the hot plate test reflected a lack of affinity for the receptors mediating this response, the compounds were tested for their ability to act as antagonists in this model. Figure 2 illustrates a typical Schild plot for the antagonism of morphine and EKC by nalorphine. Results for the other drugs are summarised in Table 2. Of the compounds tested, all antagonized the antinociceptive action of morphine, and all but nalbuphine reversed the effect of EKC. With the exception of nalbuphine, all the agonist-antagonists produced Schild plots with slopes which did not deviate significantly from unity. The results are thus consistent with a competitive antagonism of both EKC and morphine and thus indicate that the agonist-antagonists have affinity for the receptors through which both morphine and EKC produce their antinociceptive action.

Influence of stimulus intensity

The potent antagonist properties of the agonist-antagonist analgesics indicates that their failure to produce an antinociceptive effect is unrelated to their receptor affinity, but may represent a lack of efficacy. If this were the case they would be expected to be most effective against a mild stimulus, and to display

Table 2 Antagonist potencies (AD_2 doses) for a series of opioids against morphine and ethylketocyclazocine in the mouse hot plate assay

	Morphine	*Ethylketocyclazocine*
Xorphanol	0.13 (0.057–0.29)	0.10 (0.06–0.17)
Naloxone	0.037 (0.019–0.059)	0.041 (0.02–0.086)
Levallorphan	0.15 (0.094–0.23)	0.15 (0.048–0.46)
Nalbuphine	0.29*	NT
Nalorphine	0.13 (0.032–0.51)	0.53 (0.37–0.77)
Cyclazocine	0.073 (0.059–0.089)	0.28**
Naltrexone	0.0058 (0.0034–0.0091)	0.038 (0.021–0.066)
Bremazocine	0.07 (0.04–0.12)	0.05 (0.02–0.12)

* Slope significantly different from 1.
** Extrapolated from 2 points due to behavioural effect of higher doses.
NT: not tested.

a ceiling effect against more intense stimuli. To test this hypothesis, the effect of varying the stimulus (acetic acid concentration) in the abdominal constriction model was investigated.

It is clear from Figure 3 that increasing the concentration of acetic acid had little influence on the potencies of the μ-agonist morphine or the κ-agonist tifluadom. In contrast, the agonist-antagonist analgesics became less active when the acetic acid concentration was increased to 0.6%. Furthermore, with many of these compounds the dose-response curves were markedly flattened at the higher level of stimulus. This was particularly noticeable in the case of nalorphine, nalbuphine and levallorphan, which failed to inhibit completely the response produced by 0.6% acetic acid. In contrast, the use of 0.25% acetic acid increased the sensitivity of the assay to many of the agents tested. This was again most apparent with the agonist-antagonists nalorphine and levallorphan.

Discussion

The results from the first part of this study broadly confirm those of other groups. In accord with Taber *et al.* (1964), Smits & Takemori (1970) and Tyers (1980) the present findings confirm the ability of the agonist-antagonist analgesics to inhibit the abdominal constriction response in mice. The failure of these agents to increase reaction time in response to a 55°C thermal stimulus has also been previously reported. For example, Tyers (1980, 1982) reported that nalorphine and bremazocine were ineffective in the hot plate model whilst buprenorphine yielded a bell-shaped dose-response curve with a low maximum effect. Similarly Zimet *et al.* (1986) found pentazocine and nalbuphine to be essentially inactive in the same assay. Using the tail immersion test Luttinger (1985) reported pentazocine and nalorphine to be almost devoid of activity even at doses as high as $30\,mg\,kg^{-1}$, whilst Sewell & Spencer (1976) obtained similar results with cyclazocine and pentazocine.

The finding that the agonist-antagonist analgesics block the effects of morphine and EKC in the hot plate assay confirms earlier reports that these agents are partial agonists at both the μ- and κ-receptors in isolated tissue (Kosterlitz & Waterfield, 1975; Miller *et al.*, 1986) and animal models (Tyers, 1982; Leander, 1983b).

The apparently contradictory observation that the agonist-antagonists have potent antinociceptive properties in the abdominal constriction model, but antagonist activity in the hot plate assay gives rise to two possibilities. Firstly, heat and non-heat sensations could involve different neuronal pathways containing different opioid receptor populations. Tyers (1980) has proposed that the agonist-antagonist group of analgesics produce their effects primarily at the κ-receptor, and that heat stimuli are insensitive to agents acting at this site. Alternatively, the different sensitivities of the two models could be a reflection of the intensity of the stimulus employed. The greater the stimulus, the lower would be the apparent receptor reserve of the assay such that only full agonists would be capable of generating a response.

If the first of these explanations were correct then κ-agonists should be inactive against heat stimuli. In the present study the κ-agonists EKC and tifluadom produced potent effects in the hot plate assay. However, both of these agents have been reported to have affinity for the μ-receptor (Carroll *et al.*, 1984), and since all of the μ-agonists examined were effective in the hot plate model, it could be argued that the effects of EKC and tifluadom are mediated by the μ-receptor. The finding that similar doses of naloxone were required to antagonize EKC and the μ-agonist morphine adds further weight to this contention. However, a second antagonist, naltrexone antagonized morphine at doses substantially below those required to reverse the effect of EKC (Table 2), suggesting that the two agonists produce their effects at different receptors. The other antagonists tested display similar affinities for μ- and κ-receptors in isolated tissues (Miller *et al.*, 1986) and would not therefore be expected to distinguish between morphine and EKC in the current study.

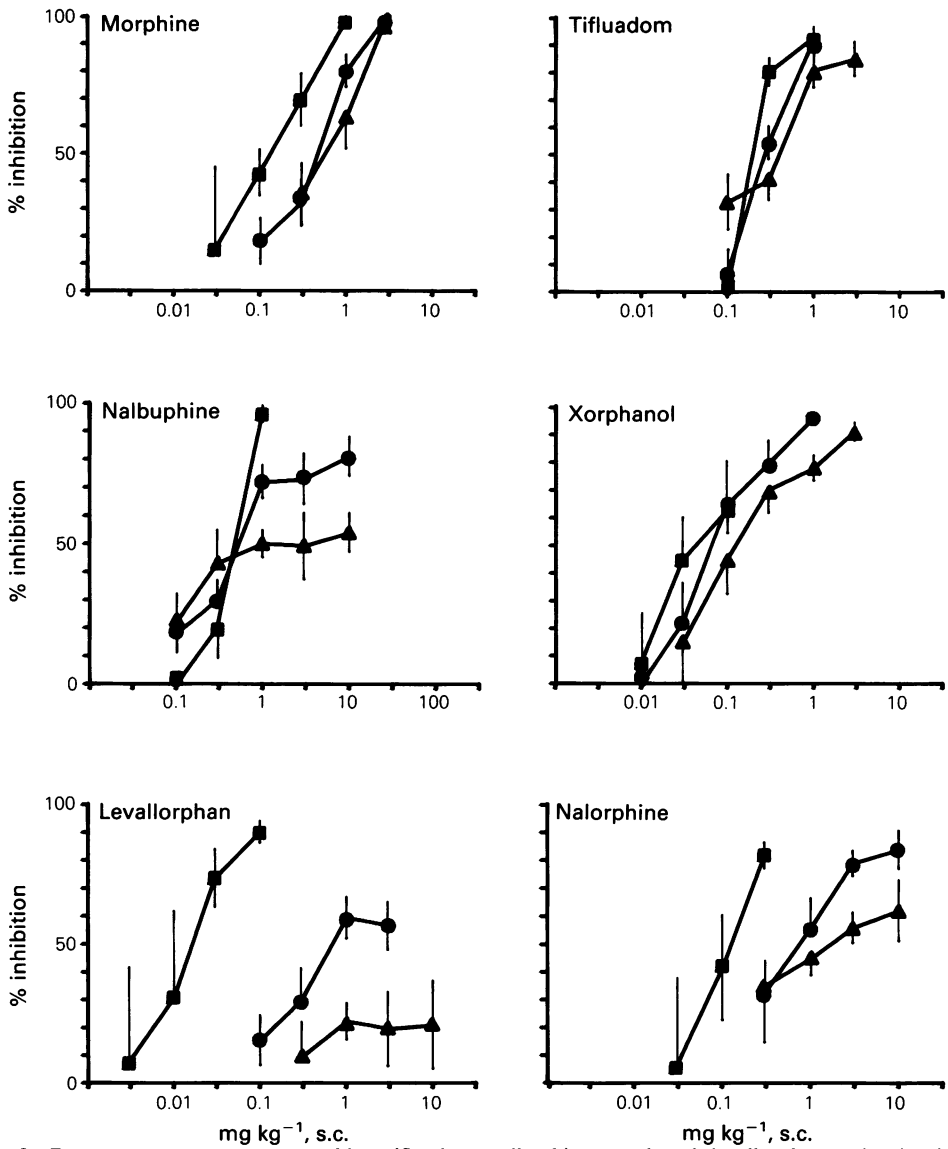

Figure 3 Dose-response curves to morphine, tifluadom, nalbuphine, xorphanol, levallorphan and nalorphine in abdominal constriction models employing 0.25% (■), 0.4% (●) and 0.6% (▲) acetic acid. Each point represents the mean of between 10 and 20 mice; s.e.mean indicated by vertical lines.

More conclusive evidence for the involvement of κ-receptors in heat models is provided by the κ-selective agent U-50488H. This compound had activity in the hot plate model at doses which, although high, were entirely consistent with its weak activity (relative to EKC and tifluadom) in isolated tissue preparations (Miller *et al.*, 1986). Similar findings have been reported by VonVoigtlander *et al.* (1983) who found U-50488H to be effective in a radiant heat tail flick model in the mouse and in a 54.5°C hot plate test in the rat, and by Delaney *et al.* (1986) who found U-50488H to be equipotent in the rat paw pressure and radiant heat tail flick assays. In a further study, Upton *et al.* (1983) found that both μ- and κ-agonists were effective in a tail immersion assay, although the latter agents were relatively more

potent in a tail pressure model. Overall, it is not possible to discount entirely the possibility that the μ-activity of some compounds (e.g. EKC) contributes to their antinociceptive potency. However, it is difficult to escape the conclusion that κ-agonists are effective against heat stimuli in several animal models.

The second explanation, that the intensity of the noxious stimulus determines the sensitivity of a test to partial agonists, is supported by a number of published studies. Several workers have shown that reducing the intensity of the noxious stimulus greatly enhances the sensitivity of antinociceptive tests to the agonist-antagonist compounds. For example, Luttinger (1985) has reported that pentazocine and nalorphine, which were undetectable in a 55°C tail immersion test, produced large increases in the time taken to react to a 45°C stimulus. Similar findings were reported by Gray *et al.* (1970) and by Granat & Saelens (1973) using radiant heat tail flick assays. The results from the present study, in which an increase in the concentration of acetic acid diminished the apparent efficacy of agents such as nalorphine and levallorphan, add further weight to this hypothesis.

It would thus appear that the sensitivity of animal models of antinociceptive activity can be manipulated by changing the intensity of the noxious stimulus, and that this applies not only to thermal but also to chemical stimuli. Since the results of this and many previous studies can be explained by the efficacy argument alone, and there are several reports of κ-agonists inhibiting responses to noxious heat, the available evidence tends to support the second of the two hypotheses.

If it is accepted that the major factors determining the action of an analgesic are the intrinsic activity of the drug and the intensity of the noxious stimulus (i.e. the apparent receptor reserve of the test) then several important points emerge. Firstly, these findings highlight the need for caution when comparing data from different laboratories since the same test is seldom conducted under identical conditions. For example, the acetic acid abdominal constriction model is widely used, but the concentration of acetic acid varies from 0.25% to as high as 19% (Drower *et al.*, 1987). In view of the change in sensitivity observed over the narrow concentration-range of the present study, more concentrated acid would be expected to produce an extremely insensitive assay. This is illustrated by the finding of Schmauss *et al.* (1983) that the use of 9% acetic acid produced an assay which was generally less sensitive than either the 55°C hot plate or tail flick models.

This finding is not unique to antinociceptive tests, nor even to *in vivo* studies. There are numerous reports that changing the stimulus intensity in field-stimulated isolated tissue models can have profound effects on both the sensitivity of the assay to opioid agonists, and on the ability to detect the agonist action of partial agonists (Hart *et al.*, 1979; Smith, 1984; Ramme & Illes, 1986).

Table 3 Percentage occupancy of μ- (morphine) and κ- (ethylketocyclazocine) receptors at the abdominal constriction test ED_{50} dose

	ED_{50}	% occupancy μ	% occupancy κ
Bremazocine	0.01	12.5	16.7
Xorphanol	0.08	38.5	43.5
Cyclazocine	0.23	75.9	45.1
Nalorphine	0.77	85.7	59.2
Levallorphan	1.1	88.1	88
Nalbuphine	1.4	82.8	ND

Occupancy values calculated as $ED_{50} \div (ED_{50} + AD_2)$.
ND: not determined.

An unusual feature of the present study is that it provides a measure of both agonist and antagonist potency for a range of partial agonists. Since both the abdominal constriction ED_{50} values and the hot plate AD_2 values were obtained in the same species and after the same time and route of administration, it is possible to use the data to estimate the intrinsic activities of the drugs tested.

According to the Langmuir equation:

$$\text{occupancy} = \frac{\text{concentration}}{\text{concentration} + \text{affinity}}$$

In the present study, concentration translates to ED_{50} dose, whilst affinity is equivalent to the AD_2 value. Clearly, the estimates of ED_{50} and AD_2 are likely to be influenced by pharmacokinetic factors. However, since both assays were conducted in the same species, with identical dosing protocols, any correction factor would apply equally to both of the measured values and would cancel out in the Langmuir equation. It is thus possible to calculate for each partial agonist the receptor occupancy required to achieve a 50% inhibition of the abdominal constriction response (Table 3). It is apparent from these results that at equianalgesic doses, bremazocine occupies only 16% of available μ- and κ-receptors whilst levallorphan occupies >80%. This implies that the intrinsic activity of levallorphan is substantially lower than that of bremazocine, whilst the other drugs have intermediate values. It is interesting to note that the two drugs which require the greatest receptor occupancy to achieve analgesia, nalorphine and levallorphan, are also the drugs which are most influenced by changes to the acetic acid concentra-

tion in the abdominal constriction model. This close agreement between the two methods of estimating intrinsic activity adds further weight to the validity of this approach.

A final point to emerge from the study concerns the relevance of many animal antinociceptive models. The use of, for example, a 55°C thermal stimulus or 9% acetic acid as a chemical stimulus yields models which, although retaining the ability to detect morphine, are insensitive to many analgesics such as nalbuphine, buprenorphine and pentazocine. Since all these drugs have clinically-proven efficacy, it implies that the stimuli employed in such tests exceed those encountered in clinical pain, and must raise serious doubts about the predictive value of these assays.

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