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Analgesic doses of the enkephalin degrading enzyme inhibitor RB 120 do not have discriminative stimulus properties

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Abstract

The systemically active mixed inhibitor of enkephalin metabolism, *N*-((*S*)-2-benzyl-3[(*S*) 2-amino-4-methylthio)butyldithio-]-1-oxopropyl)-L-alanine benzylester (RB 120), alone or in combination with 4-{[2-[[3-(1*H*-indol-3-yl))-2-methyl-1-oxo-2-[[(tricyclo-[3.3.1.1.]dec-2-yloxy) carbonyl]amino}propyl]amino]-1-phenylethyl]amino}-4-oxo-[*R*-(*R**,*R**)]-butanoate *N*-methyl-D-glucamine (CI 988; CCK_1 receptor antagonist) was investigated for discriminative and morphine generalisation effects using an operant drug discrimination paradigm in rats. Animals dosed with RB 120 (10 mg/kg) failed to develop a discriminative response. Combined CI 988 (0.3 mg/kg) and RB 120 (10 mg/kg) also failed to elicit a discriminative response. Morphine-trained animals (3.0 mg/kg) did not generalise to RB 120 (10 and 20 mg/kg). Similarly, subsequent retraining of the same animals with 1.5 mg/kg of morphine did not elicit generalisation to RB 120 (10 or 20 mg/kg). Combined RB 120 (10 or 20 mg/kg) and CI 988 (0.3 or 3.0 mg/kg) treatment produced no notable drug lever selection in rats able to discriminate morphine (1.5 mg/kg) from saline. These results suggest that RB 120 may have low abuse potential at analgesic doses. © 2000 Published by Elsevier Science B.V.

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1. Introduction

Rather than using drugs to directly stimulate opioid receptors, an alternative pharmacological approach can be adopted. This involves the use of agents that inhibit endogenous opioid degrading enzymes thereby facilitating opioidergic neurotransmission indirectly. The two enzymes responsible for degradation of enkephalins in the brain are aminopeptidase N (APN, E.C.3.4.11.2) and neutral endopeptidase (NEP, E.C.3.4.24.11) (for review, see Roques et al., 1993). Inhibition of both enzymes in vivo by (*N*-(*R*,*S*)-2-benzyl-3[(*S*)(2-amino-4-methylthio)butyldithio-]-1-oxo-propyl-L-phenylalanine benzylester (RB 101), a mixed inhibitor pro-drug able to cross the blood brain barrier (Fournié-Zaluski et al., 1992), produces naloxone-reversible analgesia (Noble et al., 1992b). More recently, *N*-((*S*)-2-benzyl-3[(*S*) 2-amino-4-methylthio)-butyldithio-]-1-oxopropyl)-L-alanine benzylester (RB 120), a novel structural analogue of RB 101, has been synthesised, showing for the first time for such a dual peptidase inhibitor, strong analgesic effects in mice and rats after oral administration (Noble et al., 1997).

The ability of morphine and other mu opioid receptor agonists to act as discriminative cues in rats is well established (Colpaert et al., 1976, 1978a,b; Colpaert, 1978; Joharchi et al., 1993). Animals can be trained in a two lever choice task to select an appropriate lever depending on whether they have been administered morphine or saline before the test session (Colpaert, 1978). Subsequently, novel drugs can be tested for their ability to replace, or generalise to, the trained drug cue expressed by responding on the appropriate lever. In this context, intracerebral administration of the selective neutral endopepti-

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dase inhibitor thiorphan (Roques et al., 1980) does not generalise to a morphine discriminative stimulus, neither does it enhance the morphine cue (Buxton et al., 1982). Furthermore, this inability to generalise to a morphine cue was also observed for both the systemically administered thiorphan prodrug acetorphan (Knisely et al., 1989) and the orally active neutral endopeptidase inhibitor [(*S*)-*N*-N-1-(2,2-dimethyl-1,3-dioxolan-4yl)methoxycarbonyl-2-phenylethyl-1-phenylalanine-β-alanine (SCH34826; Knisely et al., 1989). Several opioid peptides and peptide analogues such as D-Ala^2, D-Leu^5-enkephalin and D-Ala^2, *N*-Me-Phe^4, Gly-ol^5)-enkephalin produce morphine-like discriminative stimulus effects (Locke and Holtzman, 1986). However, central administration of met-enkephalin did not result in morphine generalisation and this lack of effect was attributed to rapid inactivation of the peptide (Colpaert et al., 1978b). Mixed enkephalin catabolism inhibitors, such as RB 101, produce more intense pharmacological and biochemical responses than compounds inhibiting only neutral endopeptidase which offer partial protection of endogenous enkephalins (Fournié-Zaluski et al., 1984; Roques et al., 1993). This suggests that the lack of morphine generalisation effects of neutral endopeptidase inhibitors may relate to lower pharmacological potency. Therefore, in this study we have investigated the effects of RB 120 for discriminative and morphine generalisation effects to determine whether combined inhibition of aminopeptidase N and neutral endopeptidase induces an interoceptive cue.

The enhancing effects of CCK_1 receptor antagonists on morphine induced analgesia (Dourish et al., 1990) and reward (Higgins et al., 1992) have also been investigated using RB 101. Thus, significant potentiation of the analgesic effects of RB 101 was observed following pre-treatment with the selective CCK_1 receptor antagonists 3*R*-(+)-*N*-(2,3-dihydro-1-methyl-2-oxo-5 phenyl-1*H*-1,4-benzodiazepine-3-yl)-*N*′-(3-methyl phenyl)-urea (L-365,260; Maldonado et al., 1993) and 4-{[2-[[3-(1*H*-indol-3-yl)2-methyl-1-oxo-2-[[(tricyclo[3.3.1.1.$^{3.7}$]dec-2-yloxy)carbonyl]amino]propyl]amino]-1-phenylethyl]amino}-4-oxo-[*R*-(*R**, *R**)]-butanoate *N*-methyl-D-glucamine (CI 988) (Valverde et al., 1994) in rodents. Similarly, Valverde et al. (1995b) observed facilitation of RB 101 rewarding effects following pre-treatment with CI 988 in the place preference paradigm. CI 988 has also been shown to enhance the ability of RB 101 to attenuate the naloxone-induced morphine withdrawal syndrome (Maldonado et al., 1995). The combination of CCK_1 receptor antagonists and mixed inhibitors of enkephalin degradation has therefore been proposed as a potential strategy in pain relief management (Maldonado et al., 1993; Roques et al., 1993). However, since this combination results in an enhancement of endogenous enkephalins and since these effects are mediated via opioid receptors, the question of possible abuse liability of such a drug combination has arisen (Maldonado et al., 1995).

The acquired discrimination of drug cues by animals is thought to model the subjective effects of drugs in humans (Holtzman, 1990). Furthermore, the subjective effects of drug intake in humans is proposed as a major factor in explaining why drugs are abused (Stolerman, 1992). Drug discrimination therefore represents a useful method for assessing abuse liability of novel drugs that could have clinical applications (Balster, 1991; Holtzman, 1990) such as the new mixed inhibitors of enkephalin catabolism.

The aims of this study were two-fold: firstly, to determine whether the orally-active mixed inhibitor of enkephalin catabolism, RB 120 (Noble et al., 1997), could act as a discriminative stimulus either in its own right or in combination with CI 988; and secondly to determine whether RB 120 or the combination of RB 120 with CI 988 could substitute for the morphine discriminative cue.

2. Materials and methods

2.1. Subjects

Singly-housed adult male albino Wistar rats weighing 200–250 g (Cardiff University breeding stock) were employed for these studies. Animals were maintained on a restricted diet described below to induce motivation for lever responding to obtain food rewards as part of the drug discrimination paradigm. Rats were maintained at 21 ± 2°C, on a 12 h/12 h light/dark cycle (lights on 07:00 h). Experiments were performed during the light phase of the light/dark cycle. Training and test sessions were conducted under low light conditions (< 10 lx). Ventilation fans of the operant chambers generated background white noise. All animal procedures were conducted in accordance with licenses issued under the United Kingdom 1986 Animals (Scientific Procedures) Act.

2.2. Drugs

RB 120 was synthesised in the laboratory of B.P.R, as previously reported (Noble et al., 1997). Morphine hydrochloride was purchased from Martindale pharmaceuticals (UK) and CI 988 was generously provided by Professor J. Hughes (Parke-Davis Research Centre, Cambridge, UK). RB 120 and morphine were dissolved in commercial apyrogenic water (for injection) and CI 988 was suspended in an aqueous solution of methylcellulose (1%). All drugs were injected intraperitoneally using a dose volume of 1 ml/kg.

2.3. Apparatus

Four standard operant chambers (Campden Instruments, UK) fitted with two response levers positioned either side of a central food hopper were used. Two aluminium partitions extending 4 cm into the chamber were placed

between the food hopper and the levers. Access to the food was via a perspex panel flap. All four boxes were controlled by a BBC Master computer with software written by D.M.P using the SPIDER interface language developed by Paul Fray, Cambridge, UK.

2.4. Drug discrimination training

Animals were initially trained to respond to each lever presented one at a time with the required levers pressed to obtain a food pellet gradually increased from fixed ratio (FR) 1 up to FR 10. That is at FR 10, 10 presses on the presented lever were required before the presentation of a 45-mg food pellet. Once appropriate lever pressing had been established, drug discrimination training was started. This involved the presentation of both levers during a training session with one being assigned the reward lever according to whether the animal had been given drug or saline. To control for positional preference, half of the animal groups were trained to respond on the left lever following drug and half to the right. Food delivery was indicated by the illumination of a light situated behind the food panel flap. Responses on the other lever were not reinforced. The houselight was kept on for the duration of the test session and to prevent olfactory cues left by lever selection from previous animals, levers were swabbed at the end of each session with 5% Hibitane™ solution. Training sessions lasted 15 min or until 50 rewards had been obtained and were conducted each week day. Rats were maintained at 85–90% of their free-feeding body weight by restricting food intake to 15–17 g standard laboratory pellets given daily after each training session. Animals were prevented from predicting the sequence of drug and saline training sessions by using a pseudorandomised schedule (Joharchi et al., 1993). This was repeated every 20 days and was counterbalanced within each group according to the following sequence: DVVDV, VDVDD, VDDVV, DVDVD (V = vehicle, D = drug). Criterion for recognising the acquisition of a discriminative cue was set at 8 correct choices in 10 consecutive sessions, the ''correct'' choice being said to occur if no more than 16 lever presses were made before the presentation of the first food pellet. Furthermore, over any given session at least 90% of the total responses must have been made on the reward lever. Once the training criteria were attained, generalisation testing began with experiments being conducted twice a week, on Tuesdays and Fridays. Normal training continued on the remaining week days. In studies where discrimination of the drug cue was not acquired, training was discontinued after 80 training sessions.

The dose of morphine used in the study has previously been shown to produce good discriminative cue properties (Joharchi et al., 1993) while the dose of RB 120 has similar analgesic efficacy to the corresponding dose of morphine (Noble et al., 1997). The CI 988 dose used and its pre-treatment time are identical to those previously shown to enhance the rewarding effects of RB 101 (Valverde et al., 1995b).

2.5. Drug discrimination generalisation testing

All rats from tested groups were used for generalisation testing but only data from animals at the set criteria for drug cue discrimination on the day of generalisation testing were analysed. On test days, both levers were initially programmed to provide reinforcement. However, the first lever pressed 10 times resulted in a delivery of a food pellet and became the chosen lever. Subsequently, responding on the other lever was unrewarded. Further responding on the chosen lever resulted in food delivery up to the provision of five pellets (50 lever presses), each test session lasting no more than 5 min. The sequence of drug and dose used in the generalisation testing was randomised and conducted on the second and fifth day of each 5-day training week. Parameters for test days included the number of animals responding, the number choosing the drug lever, percentage drug lever selection and the response rate (total number of lever presses/test session duration). For this latter parameter, subjects not finishing within 5 min were given the default session duration time of 300 s for analysis purposes.

2.6. Experiment 1 — discrimination training with morphine and RB 120

One group of 10 animals was trained to discriminate morphine (3 mg/kg) from vehicle employing a pre-treatment time of 10 min prior to each training session. A second group ($n = 10$) received either RB 120 (10 mg/kg) or vehicle, also 10 min before each session. Once discrimination had been achieved the morphine group underwent generalisation testing (see below). This was followed by a period of re-training to a new dose of morphine (1.5 mg/kg) and an extended pre-treatment time (20 min). Reinforcement lever designations remained unchanged.

2.7. Experiment 2 — discrimination training with RB 120 following CI 988 pre-treatment

After 80 training sessions, a 30 min CI 988 (0.3 mg/kg) pre-treatment time was added to the training schedule of those animals being treated with RB 120 (10 mg/kg; $n = 6$). On non-drug training days, animals were pre-treated with CI 988 vehicle (1% w/v methylcellulose) 20 min before being given the RB 120 vehicle. The appropriate reward lever for drug or vehicle was reversed for each animal at the start of the new training schedule in order to minimise the risk of false positives and be confident that any discriminative cue observed was genuine and robust.

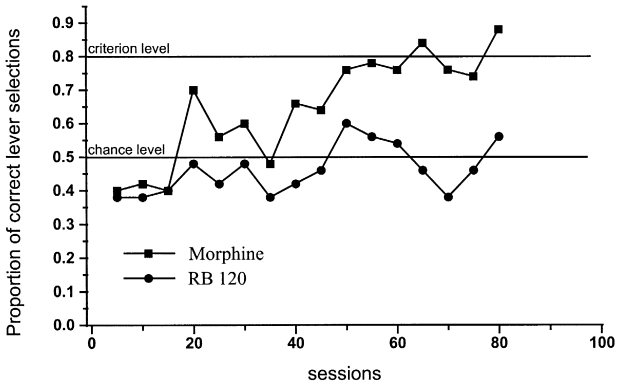

Fig. 1. Experiment 1: Acquisition of discriminative stimulus to 3.0 mg/kg morphine (filled squares) and not 10 mg/kg RB 120 (filled circles). Discrimination is expressed as mean proportion of correct lever selections per 5-day training week ($n = 10$). The chance level line indicates lever selection obtained by chance. The criterion line indicates the attainment of correct drug lever selection as defined in Section 2.

2.8. Experiment 3 — generalisation testing with rats trained to discriminate morphine (3.0 mg / kg)

Generalisation tests in the group trained to discriminate morphine (3.0 mg/kg) were conducted with morphine doses of 0, 0.15, 0.75, 2.25, 3.0 and 3.75 mg/kg and RB 120 at 5, 10 and 20 mg/kg.

2.9. Experiment 4 — generalisation testing with rats trained to discriminate morphine (1.5 mg / kg)

Generalisation tests were conducted with morphine (0, 0.075, 0.75, 1.5, 1.875, 3.0 mg/kg) in the morphine 1.5 mg/kg trained group. The ability of RB 120 (10 and 20 mg/kg) or CI 988 (0.3, 1.0 and 3.0 mg/kg) to cause morphine lever selection was also tested. The combination of RB 120 pre-treated with CI 988 was also tested using RB 120 (10 mg/kg) + CI 988 (0.3 mg/kg), RB 120 (20 mg/kg) + CI 988 (0.3 mg/kg) and finally RB 120 (20 mg/kg) + CI 988 (3.0 mg/kg). All CI 988 doses were administered 20 min before RB 120.

2.10. Data analysis

Parameters recorded included the number of animals responding, the number choosing the drug lever, percentage drug lever selection and the response rate (total number of lever presses/test session duration). For this latter parameter, subjects not finishing within 5 min were given the default session duration time of 300 s for analysis purposes. Generalisation was said to have occurred if 80% of subjects chose the drug-appropriate lever during test sessions, although a lever discrimination index was also used to determine the tendency for the training drug to generate an interoceptive cue or for a test treatment to generalise to the training drug. This was taken as the mean ± S.E.M. number of responses on the vehicle lever before the drug lever was pressed 10 times. During test sessions, the first lever pressed 10 times became the active lever. Thus, animals selecting the vehicle lever first scored 10 and those making 10 lever presses on the drug lever exclusively first scored 0. All data was analysed using one-way analysis of variance (ANOVA) with Dunnett's post hoc test when appropriate.

3. Results

3.1. Experiment 1 — discrimination training with morphine and RB 120

Animals dosed with morphine (3.0 mg/kg) acquired the discriminative cue in 48.4 ± 5.1 sessions (Fig. 1) and were subsequently used for generalisation testing (see experiment 3). Continuation of training in the same subjects at the lower dose of morphine (1.5 mg/kg) showed acquisition of discrimination in 15.8 ± 2.6 sessions (data not shown) and were again used for generalisation testing (see experiment 4). No discriminative cue was acquired in rats dosed with RB 120 (10 mg/kg) or vehicle, even after 80 training sessions. All subjects attained the maximum number of rewards during training sessions. No significant effect on the lever response rate was observed.

3.2. Experiment 2 — discrimination training with RB 120 pre-treated with CI 988

Animals pre-treated with CI 988 (0.3 mg/kg) then given RB 120 (10 mg/kg) failed to show any discrimination to these treatments after 80 training sessions (Fig. 2). All subjects received the maximum number of rewards during training sessions. No significant effect on the lever response rate was observed.

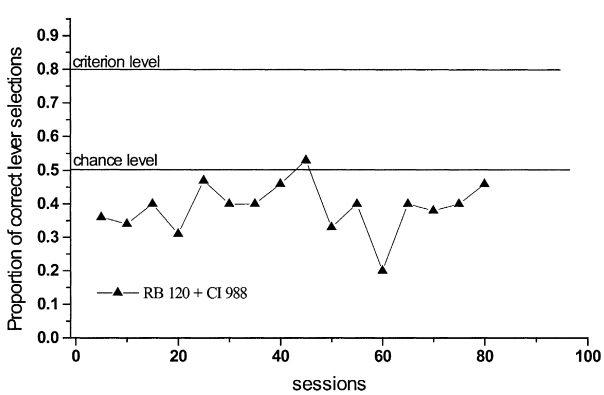

Fig. 2. Experiment 2: Acquisition of drug discrimination expressed as mean proportion of correct lever selections per 5-day training week, $n = 6$ per group. Training dose of RB 120 (10 mg/kg) pre-treated with CI 988 (0.3 mg/kg). The chance level line indicates lever selection obtained by chance. The criterion line indicates the attainment of correct dug lever selection as defined in Section 2.

Table 1
Generalisation results for rats discriminating morphine 3.0 mg/kg

Drug	Dose (mg/kg)	(1) Number responding	(2) Number choosing drug lever	(3) Percentage drug lever selection	(4) Lever discrimination index	(5) Response rate
Morphine	0.0	7/7	0/7	0.0	10.0 ± 0.0	3.6 ± 0.4
	0.15	6/6	2/6	33.3	6.7 ± 1.9	5.0 ± 1.0
	0.75	6/6	3/6	50.0	5.0 ± 2.0	4.1 ± 0.8
	2.25	7/7	5/7	71.4	2.9 ± 1.7[a]	6.7 ± 2.1
	3.0	6/6	4/6	66.7	3.3 ± 1.9[a]	5.1 ± 1.9
	3.75	7/7	7/7	100.0	0.0 ± 0.0[a]	2.6 ± 0.5
RB 120	0.0	8/8	0/8	0.0	10.0 ± 0.0	3.7 ± 0.4
	5	9/9	0/9	0.0	10.0 ± 0.0	2.7 ± 0.3
	10	8/8	1/8	12.5	8.75 ± 1.2	2.4 ± 0.4
	20	5/7	0/5	0.0	10.0 ± 0.0	2.9 ± 1.0

(1) Number of animals obtaining at least one reward. (2) Number of animals obtaining a reward from the drug assigned lever. (3) Percentage of animals in that session selecting the drug lever. (4) Mean (±S.E.M.) number of responses on vehicle lever before drug lever was pressed 10 times. The first lever pressed 10 times became the active lever. Thus, animals selecting the vehicle lever first scored 10 and those making 10 lever presses on the drug lever exclusively first scored 0. (5) Mean (±S.E.M.) response rate (total number of lever responses divided by time taken to reach five rewards or test session length).

[a] $P < 0.05$ (one-way ANOVA with Dunnett's post hoc test).

3.3. Experiment 3 — generalisation testing with rats trained to discriminate morphine (3.0 mg / kg)

Results of the generalisation study show a dose-dependent increase in morphine lever selection (Table 1). The training dose of morphine caused 66.7% of animals to select the morphine associated lever, while 3.75 mg/kg increased response accuracy to 100%. Administration of RB 120, however, induced no notable drug lever selection ($F[3,28] = 0.41$) at any of the doses used (Table 1). After administration of RB 120 (20 mg/kg), two animals, out of the seven eligible for data analysis, failed to respond on either of the presented levers. The remaining five individuals selected the vehicle lever and obtained the maximum

Table 2
Generalisation results for rats discriminating morphine 1.5 mg/kg

Drug	Dose (mg/kg)	(1) Number responding	(2) Number choosing drug lever	(3) Percentage drug lever selection	(4) Lever discrimination index	(5) Response rate
Morphine	0.0	7/7	0/7	0.0	10.0 ± 0.0	4.0 ± 2.1
	0.075	6/6	0/6	0.0	10.0 ± 0.0	2.6 ± 0.7
	0.75	7/7	4/7	57.1	4.4 ± 1.9[a]	3.0 ± 0.9
	1.5	7/7	5/7	71.4	5.4 ± 1.8[a]	2.5 ± 0.9
	1.875	7/7	5/7	71.4	5.4 ± 1.8[a]	1.8 ± 0.4
	3.0	6/6	6/6	100.0	1.0 ± 0.5[a]	3.2 ± 0.9
RB 120	10.0	6/6	0/6	0.0	10.0 ± 0.0	2.9 ± 1.0
	20.0	6/6	0/6	0.0	10.0 ± 0.0	2.4 ± 0.6
CI 988	0.3	6/6	0/6	0.0	10.0 ± 0.0	3.1 ± 1.0
	1.0	7/7	0/7	0.0	10.0 ± 0.0	3.3 ± 0.7
	3.0	7/7	0/7	0.0	10.0 ± 0.0	2.6 ± 0.6
RB 120 + CI 988	10.0 0.3	6/6	1/6	16.7	8.3 ± 1.7	6.2 ± 2.3
RB 120 + CI 988	20 0.3	6/6	1/6	16.7	9.8 ± 0.2	3.4 ± 0.7
RB 120 + CI 988	20 3.0	7/7	1/7	14.3	8.6 ± 1.4	3.2 ± 0.8

(1) Number of animals obtaining at least one reward. (2) Number of animals obtaining a reward from the drug assigned lever. (3) Percentage of animals in that session selecting the drug lever. (4) Mean (±S.E.M.) number of responses on vehicle lever before training drug lever was pressed 10 times. The first lever pressed 10 times became the active lever. Thus, animals selecting the vehicle lever first scored 10 and those making 10 lever presses on the training drug lever exclusively first scored 0. (5) Mean (±S.E.M.) response rate (total number of lever responses divided by time taken to reach five rewards or session length).

[a] $P < 0.05$ (one-way ANOVA with Dunnett's post hoc test).

number of rewards for the session. No significant effect was observed on response rate.

3.4. Experiment 4 — generalisation testing with rats trained to discriminate morphine (1.5 mg/kg)

Since administration of RB 120 resulted in no significant drug lever selection in rats trained to discriminate morphine (3.0 mg/kg) and vehicle, the sensitivity of these rats to stimulus generalisation was augmented by continued training at a lower morphine dose (1.5 mg/kg). Rats trained to this modified discriminative morphine stimulus showed a dose-dependent generalisation to increasing doses of morphine during test sessions (71.4% of animals chose the drug lever at the training dose of 1.5 mg/kg, while 100% chose the drug lever at 3.0 mg/kg — Table 2). Administration of either RB 120 ($F[2,16] = 0.00$) or CI 988 ($F[3,23] = 0.00$) did not cause any drug lever selection and had no effect on the response rate parameter. Similarly, no significant drug lever selection was noted for the combination of RB 120 with CI 988, although one individual from each drug combination selected the drug lever ($F[3,21] = 0.32$). A slight increase in the response rate of those animals given the combination of RB 120 (10 mg/kg) and CI 988 (0.3 mg/kg) was observed, but this was not significant ($F[3,23] = 0.7$). Thus, administration of RB 120, CI 988 or the combination of these drugs induced no notable drug lever selection but in all cases animals obtained the maximum number of rewards possible in the test session.

4. Discussion

Under the experimental conditions used in this study (experiment 1) RB 120, unlike morphine, does not generate a cue to indicate lever selection even after extended periods of training (Fig. 1). Close examination of the data for each animal also revealed that no individual assigned to the RB 120 group had any tendency towards acquiring an interoceptive cue. Administration of the CCK_1 receptor antagonist CI 988 before RB 120, in experiment 2, also failed to generate a stimulus cue, despite extending the number of training sessions (Fig. 2). Since the maximum number of rewards was obtained before the end of the each session, it may be deduced that the ability to perform the task was not impaired. The dose of RB 120 selected (10 mg/kg) produces analgesic responses similar to the morphine dose (3.0 mg/kg) in a variety of nociceptive tests in rats (Noble et al., 1992b, 1997). Therefore, the training doses of RB 120 and morphine used in this discrimination study are pharmacologically comparable. This is of particular importance since Colpaert et al. (1978a) has shown a direct relationship between analgesic potency and strength of stimulus generalisation with opioid drugs. Moreover, the dose of CI 988 (0.3 mg/kg) has previously been reported to markedly potentiate RB 101 analgesia (Valverde et al., 1994) and reward in the rat (Valverde et al., 1995b).

The results of generalisation testing described in experiments 3 and 4 (Tables 1 and 2) also suggest that analgesic doses of RB 120 do not result in morphine lever selection even in the group of rats trained to discriminate morphine at the lower dose. It is noteworthy, however, in animals trained to discriminate 3.0 mg/kg morphine from saline (experiment 3), 2 of the 7 individuals, at the required level of discriminative ability, did not respond on either of the presented levers after RB 120 (20 mg/kg) administration. The remaining individuals completed the test session. The same dose of RB 120 in animals trained to differentiate 1.5 mg/kg morphine from saline (experiment 4) did not result in response failure, possibly due to the extended pre-treatment time used (30 instead of 10 min). We therefore consider that this mild suppression of normal responding is unlikely to have interfered with lever selection. No other dose of RB 120, CI 988 or their combinations affected the ability of animals to reach the maximum number of rewards for any given training session. The discriminative stimulus to morphine was dose dependent thereby validating the stimulus generalisation methodology. Accordingly, the lever discrimination index (otherwise known as ''error score analysis'') revealed a significant increase in drug lever selection at doses below the training dose in both generalisation experiments emphasising the sensitivity of this paradigm to drug transfer effects.

Singh et al. (1996) reported that CI 988 (0.1 to 1 mg/kg) did not induce morphine lever selection when given on its own in rats trained to discriminate morphine (3.0 mg/kg) and neither did it modify the morphine stimulus. Furthermore, the current study indicates that CI 988 at higher dose levels (3.0 mg/kg) does not cause morphine generalisation in rats trained with a lower morphine dose (1.5 mg/kg). In this context another CCK_1 receptor antagonist, L-365,260, also failed to exhibit any ability to generalise to the morphine cue or generate an interoceptive stimulus after extended training in rats (Jackson et al., 1994), a finding substantiated by Higgins et al. (1994) who reported that L-365,260 pre-treatment did not alter the morphine cue.

A possible therapeutic strategy to reduce side effects of chronic opioids is the use of CCK_1 receptor antagonists in combination with morphine-like agents (Dourish et al., 1990; Dray and Urban, 1996). However, since mixed inhibitors of enkephalin degradation, such as RB 101, are devoid of morphine-like effects such as tolerance (Noble et al., 1992c) and have weak reinforcing (Valverde et al., 1995b) and dependence inducing properties (Maldonado et al., 1990; Noble et al., 1992a), the substantial potentiation of their analgesic effects by pre-treatment with CCK_1 receptor antagonists has therefore been proposed for clinical pain relief therapy (Maldonado et al., 1993). Moreover, the facilitatory analgesic effects of CI 988 and RB 101 in

combination show weak tolerance after repeated co-administration (Valverde et al., 1995a).

This study has demonstrated that RB 120, or combined RB 120/CI 988 treatment, does not generate a discriminative stimulus or display morphine generalisation effects. This inability to produce a discriminative cue may be due to low tonic release of enkephalins in brain regions where activation of opioid receptors produces an interoceptive stimulus (Williams et al., 1987; Dauge et al., 1992). Thus, although studies indicate that exogenous administration of opioid peptides generalise to the morphine cue (Locke and Holtzman, 1986), their administration probably results in activation of all brain opioid receptors. Alternatively, RB 120 results in activation of opioid receptors only where endogenous enkephalins are tonically released. Moreover, in such regions, increased extracellular levels of endogenous enkephalins protected by dual inhibitors such as RB 101 has been shown to be insufficient to saturate all opiate receptors (Ruiz-Gayo et al., 1992; Abbadie et al., 1994). The absence of tolerance (Noble et al., 1992c), induction of mild physical dependence (Maldonado et al., 1990; Noble et al., 1992a) and weak reinforcing effects (Valverde et al., 1995b) of analgesic doses of RB 101 have been similarly explained. Furthermore, methodological differences probably account for the ability of RB 101 to induce conditioned place preference as opposed to the inability of its analogue (RB 120) to induce discrimination. Thus, certain drugs, such as morphine, cause place preference effects after limited place conditioning pairings (Mucha and Iversen, 1984) relative to the extended number of training sessions required to elicit a discriminative stimulus (Joharchi et al., 1993). However, the possibility that inadequate training accounts for the failure of RB 120 to produce an interoceptive cue is unlikely, since no tendency to discriminate a drug-induced stimulus from vehicle was expressed by any individual, despite exposure to 80 training sessions and an additional 80 sessions with CI 988 pre-treatment.

Discrimination of different classes of opioid drugs shows a close correlation to differences in subjective experiences of these drug groups in humans (Colpaert, 1977; Holtzman, 1990). Furthermore, the subjective effects of drug use in humans is proposed as a major factor in explaining why drugs are abused (Stolerman, 1992). Therefore, drug discrimination is thought to represent a practical model of human subjective experience of opioid intake (Colpaert, 1977) and a feasible method for assessing the abuse liability of novel opioid drugs. This claim may be controversial though, as some drugs with no psychoactive properties can act as a discriminative stimulus (Goudie, 1991). However, it is generally accepted that a drug that does not demonstrate discriminative properties probably indicates that the drug will not have an abuse liability (Goudie and Leathley, 1993; Hartnoll, 1991; Sanger, 1991). The predictive value of the drug discrimination procedure has been confirmed in later clinical studies for multiple compounds, including some opioids devoid of abuse liability such as loperamide (Colpaert et al., 1975; Hartnoll, 1991). On this basis, the findings of the present investigation suggest that RB 120 and its combination with CI 988 are less likely to be abused than morphine-type compounds acting directly on the mu opioid receptor.

In summary, this study demonstrates that increased enkephalin activity induced by RB 120 on its own, or in combination with the CCK_1 receptor antagonist CI 988, do not possess discriminative cue properties. The data suggests the possibility that analgesic doses of RB 120, alone or combined with CI 988 may not produce morphine-like subjective effects in humans, and that analgesic doses of RB 120 may not have morphine-like abuse potential.

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References

Abbadie, C., Honor, P., Fournié-Zaluski, M.-C., Roques, B.P., Besson, J.-M., 1994. Effects of opioids on c-Fos-like immunoreactivity induced in rat lumber spinal neurons by noxious heat stimulation. Eur. J. Pharmacol. 258, 215–227.

Balster, R.L., 1991. Drug abuse evaluation in animals. Br. J. Addict. 86, 1549–1558.

Buxton, D.A., Priestley, T., Shaw, J.S., Turnbull, M.J., 1982. Failure of the enkephalinase inhibitor thiorphan to produce the narcotic cue. Br. J. Pharmacol. 75S, 105.

Colpaert, F.C., 1977. Narcotic cue and narcotic state. Life Sci. 20, 1097–1108.

Colpaert, F.C., 1978. Discriminative stimulus properties of narcotic analgesic drugs. Pharmacol. Biochem. Behav. 9, 863–887.

Colpaert, F.C., Niemegeers, C.J.E., Lal, H., Janssen, P.A.J., 1975. Investigations on drug produced and subjectively experienced discriminative stimuli: 2. Loperamide, an antidiarrheal devoid of narcotic cue producing actions. Life Sci. 16, 717–728.

Colpaert, F.C., Kuyps, J.J.M.D., Niemegeers, C.J.E., Janssen, P.A.J., 1976. Discriminative stimulus properties of fentanyl and morphine: tolerance and dependence. Pharmacol. Biochem. Behav. 5, 401–408.

Colpaert, F.C., Niemegeers, C.J.E., Janssen, P.A.J., 1978a. Narcotic cueing and analgesic activity of narcotic analgesics: associative and dissociative characteristics. Psychopharmacology 57, 21–26.

Colpaert, F.C., Niemegeers, C.J.E., Janssen, P.A.J., Van Ree, J.M., 1978b. Narcotic cueing properties of intraventricularly administered sufentanil, fentanyl, morphine and met-enkephalin. Eur. J. Pharmacol. 47, 115–119.

Dauge, V., Kalivas, P.W., Duffy, T., Roques, B.P., 1992. Effect of inhibiting enkephalin catabolism in the VTA on motor activity and extracellular dopamine. Brain Res. 599, 209–214.

Dourish, C.T., O'Neill, M.F., Coughlan, J., Kitchener, S.J., Hawley, D., Iversen, S.D., 1990. The selective CCK-B receptor antagonist L-365,260 enhances morphine analgesia and prevents morphine tolerance in the rat. Eur. J. Pharmacol. 176, 35–44.

Dray, A., Urban, L., 1996. New pharmacological strategies for pain relief. Ann. Rev. Pharmacol. Toxicol. 36, 253–280.

Fournié-Zaluski, M.-C., Chaillet, P., Bouboutou, R., Coulaud, A., Cherot, P., Waksman, G., Costentin, J., Roques, B.P., 1984. Analgesic effects of kelatorphan, a new highly potent inhibitor of multiple enkephalin degrading enzymes. Eur. J. Pharmacol. 102, 525–528.

Fournié-Zaluski, M.-C., Coric, P., Turcaud, S., Lucas, E., Noble, F., Maldonado, R., Roques, B.P., 1992. ''Mixed inhibitor-prodrug'' as a new approach towards systemically active inhibitors of enkephalin-degrading enzymes. J. Med. Chem. 35, 2473–2481.

Goudie, A.J., 1991. Animal models of drug abuse and drug dependence. In: Willner, P. (Ed.), Behavioural Models in Psychopharmacology: Theoretical, Industrial and Clinical Perspectives. Cambridge Univ. Press, Cambridge, pp. 453–484.

Goudie, A.J., Leathley, M.J., 1993. Drug-discrimination assays. In: Sahgal, A. (Ed.), Behavioural Neuroscience: A Practical Approach vol. II Oxford Univ. Press, Oxford, pp. 145–167.

Hartnoll, R.L., 1991. The relevance of behavioural models of drug abuse and dependence liabilities to the understanding of drug misuse in humans. In: Willner, P. (Ed.), Behavioural Models in Psychopharmacology: Theoretical, Industrial and Clinical Perspectives. Cambridge University Press, Cambridge, pp. 503–519.

Higgins, G.A., Nguyen, P., Sellers, E.M., 1992. Morphine place conditioning is differentially affected by CCK_A and CCK_B receptor antagonists. Brain Res. 572, 208–215.

Higgins, G.A., Joharchi, N., Wang, Y., Corrigall, W.A., Sellers, E.M., 1994. The CCK_A receptor antagonist devazepide does not modify opioid self-administration or drug discrimination: comparison with the dopamine antagonist haloperidol. Brain Res. 640, 246–254.

Holtzman, S.G., 1990. Discriminative stimulus effects of drugs: relationship to potential for abuse. In: Adler, M.W., Cowan, A. (Eds.), Modern Methods in Pharmacology: Assessment of Physical Dependence Techniques for the Evaluation of Abused Drugs vol. 6, Wiley-Liss, New York, pp. 193–210.

Jackson, A., Tattersall, D., Bentley, G., Rycroft, W., Bourson, A., Hargreaves, R., Tricklebank, M., Iversen, S.D., 1994. An investigation into the discriminative stimulus and reinforcing properties of the CCK_B-receptor antagonist, L-365,260 in rats. Neuropeptides 26, 343–353.

Joharchi, N., Sellers, E.M., Higgins, G.A., 1993. Effect of $5\text{-}HT_3$ receptor antagonists on the discriminative stimulus properties of morphine in rats. Psychopharmacology 112, 111–115.

Knisely, J.S., Beardsley, P.M., Aceto, M.D., Balster, R.L., Harris, L.S., 1989. Assessment of the abuse potential of acetorphan, an enkephalinase inhibitor. Drug Alcohol Depend. 23, 143–151.

Locke, K.W., Holtzman, S.G., 1986. Behavioral effects of opioid peptides selective for mu or delta receptors. Morphine-like discriminative effects. J. Pharmacol. Exp. Ther. 238, 990–996.

Maldonado, R., Feger, J., Fournié-Zaluski, M.-C., Roques, B.P., 1990. Differences in physical dependence induced by selective mu and delta opioid agonists and by endogenous enkephalins protected by peptidase inhibitors. Brain Res. 520, 247–254.

Maldonado, R., Derrien, M., Noble, F., Roques, B.P., 1993. Association of the peptidase inhibitor RB 101 and the CCK-B antagonist strongly enhances antinociceptive responses. NeuroReport 4, 947–950.

Maldonado, R., Valverde, O., Ducos, B., Blommaert, A.G., Fournié-Zaluski, M.-C., Roques, B.P., 1995. Inhibition of morphine withdrawal by the association of RB 101, an inhibitor of enkephalin catabolism, and the CCK_B antagonist PD-134,308. Br. J. Pharmacol. 114, 1031–1039.

Mucha, F., Iversen, S.D., 1984. Reinforcing properties of morphine and naloxone revealed by conditioned place preferences: a procedural examination. Psychopharmacology 82, 241–247.

Noble, F., Coric, P., Fournié-Zaluski, M.-C., Roques, B.P., 1992a. Lack of physical dependence in mice after repeated systemic administration of the mixed inhibitor prodrug of enkephalin-degrading enzymes, RB101. Eur. J. Pharmacol. 223, 91–96.

Noble, F., Soleilhac, J.M., Soroca-Lucas, E., Turcaud, S., Fournié-Zaluski, M.-C., Roques, B.P., 1992b. Inhibition of the enkephalin-metabolising enzymes by the first systemically active mixed inhibitor prodrug, RB 101, induces potent analgesic responses in mice and rats. J. Pharmacol. Exp. Ther. 261, 181–190.

Noble, F., Turcaud, S., Fournié-Zaluski, M.-C., Roques, B.P., 1992c. Repeated systemic administration of the mixed inhibitor of enkephalin-degrading enzymes, RB 101, does not induce either antinociceptive tolerance or cross-tolerance with morphine. Eur. J. Pharmacol. 223, 83–89.

Noble, F., Smadja, C., Valverde, O., Maldonado, R., Coric, P., Turcaud, S., Fournié-Zaluski, M.-C., Roques, B.P., 1997. Pain-suppressive effects on various nociceptive stimuli (thermal, chemical, electrical and inflammatory) of the first orally active enkephalin-metabolizing enzyme inhibitor RB 120. Pain 73, 383–391.

Roques, B.P., Fournié-Zaluski, M.-C., Soroca, E., Lecomte, J.-M., Malfroy, B., Llorens, C., Schwartz, J.-C., 1980. The enkephalinase inhibitor thiorphan shows antinociceptive activity in mice. Nature 288, 286–288.

Roques, B.P., Noble, F., Daugé, V., Fournié-Zaluski, M.-C., Beaumont, A., 1993. Neutral endopeptidase 24.11: structure, inhibition, and experimental and clinical pharmacology. Pharmacol. Rev. 1, 87–146.

Ruiz-Gayo, M., Baamonde, A., Turcaud, S., Fournié-Zaluski, M.C., Roques, B.P., 1992. In vivo occupation of mouse brain opioid receptors by endogenous enkephalins: blockade by enkephalin degrading enzymes by RB 101 inhibits [^{3}H]diprenorphine binding. Brain Res. 571, 306–312.

Sanger, D.J., 1991. Screening for abuse and dependence liabilities. In: Willner, P. (Ed.), Behavioural Models in Psychopharmacology: Theoretical, Industrial and Clinical Perspectives. Cambridge Univ. Press, Cambridge, pp. 485–502.

Singh, L., Oles, R.J., Field, M.J., Atwal, P., Woodruff, G.N., Hunter, J.C., 1996. Effect of CCK receptor antagonists on the antinociceptive, reinforcing and gut motility properties of morphine. Br. J. Pharmacol. 118, 1317–1325.

Stolerman, I., 1992. Drugs of abuse: behavioural principles, methods and terms. Trends Pharmacol. Sci. 13, 170–176.

Valverde, O., Maldonado, R., Fournié-Zaluski, M.-C., Roques, B.P., 1994. Cholecystokinin B antagonists strongly potentiate antinociception mediated by endogenous enkephalins. J. Pharmacol. Exp. Ther. 270, 77–88.

Valverde, O., Blommaert, A.G.S., Fournié-Zaluski, M.-C., Roques, B.P., Maldonado, R., 1995a. Weak tolerance to the antinociceptive effect induced by the association of a peptidase inhibitor and a CCK_B receptor antagonist. Eur. J. Pharmacol. 286, 79–93.

Valverde, O., Fournié-Zaluski, M.C., Roques, B.P., Maldonado, R., 1995b. The CCK_B antagonist PD-134,308 facilitates rewarding effects of endogenous enkephalins but does not produce place preference in rats. Psychopharmacology 123, 119–126.

Williams, J.T., Macdonald, J.C., Christie, J., North, R.A., Roques, B.P., 1987. Potentiation of enkephalin action by peptidase inhibitors in rat locus coeruleus in vitro. J. Pharmacol. Exp. Ther. 243, 397–401.