



Pergamon

Neuropharmacology 39 (2000) 2848–2855

NEURO  
PHARMACOLOGY

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# Kappa-opioid receptor modulation of nicotine-induced behaviour

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Accepted 4 July 2000

## Abstract

The ability of  $\kappa$ -opioid receptor ligands to modulate dependence-related behavioural effects of drugs like morphine and cocaine is well documented. The present study examined the effects of  $\kappa$ -opioid agonists on nicotine-induced locomotor stimulation in rats chronically pre-exposed to nicotine (0.4 mg/kg/day). U50,488 [0.5–3 mg/kg subcutaneously (s.c.)], U69,593 [0.08–0.32 mg/kg intraperitoneally (i.p.)] and CI-977 (0.005–0.02 mg/kg s.c.) administered 30 min prior to nicotine (0.06, 0.2 and 0.4 mg/kg s.c.) dose-dependently antagonised its acute locomotor-activating effect, which was completely prevented by the highest tested dose of each agonist. Baseline activity was unaffected by the largest doses of U50,488 and U69,593, but it was reduced by 0.01 and 0.02 mg/kg of CI-977. The selective  $\kappa$ -opioid receptor antagonist nor-BNI [30  $\mu$ g intracerebroventricularly (i.c.v.)] blocked the effects of U69,593 on nicotine-induced behaviour, thus supporting the involvement of  $\kappa$ -opioid receptors in this effect. In conclusion, the activation of  $\kappa$ -opioid receptors clearly prevented nicotine-induced locomotor stimulation. The effects of at least two of the  $\kappa$ -opioid agonists were not due to a general motor suppression. It is suggested that the mechanism entails a depression of nicotine-induced increases in accumbal dopamine by these compounds. The results should encourage further research on the role of the  $\kappa$ -opioid system in the behavioural and neurochemical effects of nicotine, including those related to nicotine dependence. © 2000 Elsevier Science Ltd. All rights reserved.

**Keywords:** Nicotine;  $\kappa$ -Opioid receptors; Norbinaltorphimine; Locomotor activity; Drug interaction; Rats

## 1. Introduction

The interaction of nicotine with a range of psychoactive drugs belonging to different pharmacological classes [such as dopamine and *N*-methyl-D-aspartate (NMDA) antagonists] has been studied previously. The modulation of the behavioural effects of nicotine by these compounds helps to elucidate underlying neuropharmacological mechanisms and to explore potential ways of treating nicotine dependence. To extend this research, the present study aimed at investigating the effects of activating  $\kappa$ -opioid receptors on nicotine-induced behaviour.

Agonists at the  $\kappa$ -type of opioid receptors, like U50,488, U69,593, CI-977 or the postulated endogenous ligand dynorphin (Chavkin and James, 1982), are known to have an acutely depressant effect on dopamine con-

centrations in both the dorsal and ventral striatum (Di Chiara and Imperato, 1988a; Spanagel et al., 1990; Zaratini and Clarke, 1994). In the nucleus accumbens, which receives dopaminergic input from projections originating in the ventral tegmental area of the midbrain (VTA), this effect is thought to be mediated primarily by receptors on dopaminergic nerve terminals, via presynaptic inhibition of neurotransmitter release (Smith et al., 1992; Spanagel et al., 1992). Since the selective  $\kappa$ -receptor antagonist norbinaltorphimine (nor-BNI) has the opposite effect on accumbal dopamine (Maisonnette et al., 1994; Spanagel et al., 1992), the  $\kappa$ -opioid system has been suggested to exert a tonic control over the release of dopamine in that area of the brain.

Almost all drugs of abuse share a neurochemical action opposite in direction to the effect of  $\kappa$ -opioid receptor agonists described above: they acutely up-regulate extracellular dopamine in the nucleus accumbens (Di Chiara and Imperato, 1988b). This mechanism is generally ascribed a central role in the incentive for drug consumption, i.e., for a drug's abuse potential.

The acutely administered  $\kappa$ -agonists U50,488 and

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dynorphin A have been found to reverse the increase in accumbal dopamine concentrations induced by systemic heroin (Xi et al., 1998), and equally, U50,488 reversed this increase induced by cocaine (Maisonneuve et al., 1994). This neurochemical interaction may underlie the numerous findings in the animal literature of antagonism of dependence-related behavioural drug effects by  $\kappa$ -opioid agonists; these observations have fuelled the suggestion of a therapeutic potential of these compounds for dependence disorders. An attenuation of the rewarding effects of cocaine and morphine by a range of different  $\kappa$ -opioid agonists was reported in rats, as reflected in both the self-administration and conditioned place preference paradigms (Funada et al., 1993; Glick et al., 1995; Suzuki et al., 1992). Also, heroin self-administration behaviour, together with the concomitant increase in accumbal dopamine release, was antagonised by U50,488 (Xi et al., 1998). In rhesus monkeys, several chronically administered  $\kappa$ -agonists, although not all of the compounds examined, significantly decreased cocaine self-administration (Mello and Negus, 1998; Negus et al., 1997). There is also evidence for an antagonism of the subjective effects of morphine by  $\kappa$ -agonists in rats, as measured by the drug discrimination paradigm (Negus et al., 1990; Spanagel and Shoaib, 1994).

Similar to other drugs of abuse, acute systemic nicotine injections robustly up-regulate concentrations of extracellular dopamine in the nucleus accumbens (Di Chiara and Imperato, 1988b). Consistent with the notion that this mechanism is of central importance for a drug's abuse potential, nicotine self-administration behaviour has been shown to be markedly reduced in rats with 6-hydroxydopamine lesions of the nucleus accumbens (Corrigall et al., 1992).

Surprisingly, there seems to be only one published report in which the interaction of nicotine with  $\kappa$ -opioid receptor agonists has been investigated. The mixed  $\mu$ -opioid partial agonist and  $\kappa$ -opioid agonist cyclazocine was shown to attenuate the nicotine-induced increase in accumbal dopamine release (Maisonneuve and Glick, 1999). This may lead to expectations of a neurochemical interaction between pure  $\kappa$ -receptor agonists and nicotine similar to that described between  $\kappa$ -agonists and cocaine or morphine.

After repeated exposure, nicotine, like other psychostimulants, causes marked increases in locomotor activity. Much evidence points towards a mediation of this sensitised motor-stimulant effect by enhanced mesolimbic dopamine neurotransmission (e.g., Clarke et al., 1988; Louis and Clarke, 1998). Studies in which nicotine was applied locally suggest that both the nicotine-induced dopamine release and locomotor activation are mediated primarily by nicotinic receptors in the VTA rather than by those situated in terminal regions of the dopamine system (Nisell et al., 1994; Reavill and Stolerman, 1990).

Acute administration of the  $\kappa$ -opioid agonists U50,488 and U69,593 attenuated cocaine-induced locomotor stimulation, and the repeated coadministration of these compounds with cocaine prevented the development of locomotor sensitisation (Crawford et al., 1995; Heidbreder et al. 1993, 1995). However, the development of the sensitised motor response to nicotine was not affected by the coadministration of U69,593 (Heidbreder et al., 1995).

In the present study, we examined the interaction of the acutely administered  $\kappa$ -opioid receptor agonists U50,488, U69,593 and CI-977 with basal and nicotine-induced locomotor activity in rats that expressed sensitised locomotor-stimulant effects of nicotine. To test whether any identified interaction was  $\kappa$ -specific, the ability of the selective  $\kappa$ -opioid receptor antagonist nor-BNI to reverse it was determined.

## 2. Methods

### 2.1. Animals

Male hooded Lister rats (Harlan Olac, Bicester, UK; initially 200–250 g) were housed singly with free access to food and water in rooms maintained at  $20 \pm 1^\circ\text{C}$ , humidity  $50 \pm 10\%$ , on a 12 h light/12 h dark cycle with lights on at 7 a.m.

### 2.2. Apparatus

Locomotor activity was assessed in  $30\text{ cm} \times 30\text{ cm} \times 30\text{ cm}$  chambers constructed from clear Perspex with wire mesh floors (described by Reavill and Stolerman, 1990). Two parallel beams of infrared light were located 3 cm from the walls and 4 cm above the floor. Interruptions of light beams were recorded by a computer (Arachnid system, CENES, Cambridge).

### 2.3. Drugs

Nicotine bitartrate (BDH, Poole, GB) was dissolved in isotonic saline, and the pH was adjusted to 7 with NaOH. U50,488 (*trans*-( $\pm$ )-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide methanesulfonate; Upjohn, Kalamazoo, MI) and CI-977 (enadoline: (5*R*)-(5 $\alpha$ ,7 $\alpha$ ,8 $\beta$ )-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-4-benzofuranacetamide monohydrochloride; Parke-Davis, Cambridge, UK) were dissolved in isotonic saline. U69,593 [(5 $\alpha$ ,7 $\alpha$ ,8 $\beta$ )-(–)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-benzeneacetamide; Upjohn, Kalamazoo, MI] was dissolved in 50% propylene glycol in sterile water. Norbinaltorphimine dihydrochloride (Tocris, Bristol, UK) was dissolved in sterile water. All drugs except nor-BNI were

administered in a volume of 1 ml/kg. Doses were calculated as those of the bases.

#### *2.4. Administration of norbinaltorphimine into the lateral ventricle*

Guide cannulae were implanted under anaesthesia [a mixture of medetomidine 0.3 mg/kg and ketamine 70 mg/kg intraperitoneally (i.p.)] using a Stoelting stereotaxic frame with the incisor bar set 5 mm above the interaural line. The skull was exposed, and a small hole was drilled unilaterally. The cannula was lowered to 1 mm above the target site (coordinates relative to bregma: A, -1.6; L, 1.5; V, 4.0), and, together with four additional skull screws, was embedded in dental acrylic.

The tips of the injection cannulae (28 gauge; Plastic Products, Roanoke, VA) extended beyond the guide cannula by 1 mm. Microinjections were done in a volume of 2 µl over a 44 s period, after which the injection cannula was left in place for a further 60 s. Delivery was from Hamilton syringes mounted in an infusion pump (model 341B; Sage Instruments), connected to the injection cannula by polycarbon tubing.

#### *2.5. Experimental procedure*

##### *2.5.1. Effects of $\kappa$ -opioid agonists on nicotine-induced locomotor stimulation*

Three groups of 12 animals each were sensitised to the locomotor-activating effects of nicotine by daily injections of 0.4 mg/kg nicotine subcutaneously (s.c.) for 4 weeks. During the sensitisation phase, locomotor activity was measured for 60 min on alternate days, with rats being placed into the photocell chambers immediately after the nicotine injection.

The test phase began once sensitisation to nicotine-induced locomotor stimulation had reached a plateau. Tests were conducted every third day. On the two intervening days, rats were injected with 0.4 mg/kg nicotine and were either returned to their home cages (days following test days) or underwent 60 min locomotor recording (days preceding test days), which served to keep the animals habituated to the test environment.

The  $\kappa$ -agonists U50,488 (0.0, 0.5, 1.5 and 3.0 mg/kg s.c.), U69,593 (0.00, 0.08, 0.16 and 0.32 mg/kg i.p.) and CI-977 (0.00, 0.005, 0.01 and 0.02 mg/kg s.c.) were each tested in a separate group of 12 rats. On test days, subjects were pretreated with a dose of the respective  $\kappa$ -agonist or the appropriate vehicle 30 min prior to administration of nicotine (0.00, 0.06, 0.2 and 0.4 mg/kg s.c.) and the start of 60 min of locomotor activity recording. Over a sequence of repeated test sessions, each dose of the respective  $\kappa$ -agonist was tested against each nicotine dose in a sequence randomised individually for each animal. In the case of U50,488, the largest dose and vehicle

were tested against each dose of nicotine in a separate sequence of tests.

Data were recorded as the number of cage crosses (beam breaks followed by the interruption of the other beam), both over the entire session and for each of six 10 min intervals. This measure is thought to reflect ambulation.

##### *2.5.2. Effects of a $\kappa$ -antagonist on response to U69,593 and nicotine*

Following completion of the previous experiment, 20 of the rats that had been tested with U69,593 or CI-977 underwent intracerebroventricular (i.c.v.) catheterisation and were allowed to recover from surgery for 5 days. Rats then received daily nicotine (0.4 mg/kg s.c.) injections for a further 4 weeks. Prior to the onset of experiments, they were randomly divided into two equally sized groups with the restriction that each group contained the same number of animals previously tested with U69,593 and CI-977. One group was tested with nor-BNI and one with vehicle (sterile water). Two rats from the nor-BNI group lost their head mounts during the experiment, resulting in  $n=8$  for this group and  $n=10$  for the vehicle group.

On Monday to Thursday on each of several weeks, all rats were injected with nicotine (0.4 mg/kg s.c.) followed by locomotor recording for 1 h. On Thursdays, about 17 h prior to test sessions, nor-BNI or vehicle was infused i.c.v.. Several studies have demonstrated that a single i.c.v. infusion of nor-BNI at doses including the one utilised in this study blocks  $\kappa$ -receptors selectively for several weeks (Horan et al., 1992; Jones and Holtzman, 1992; Spanagel et al., 1994). Tests of locomotor activity were conducted on Fridays. Over four test sessions, each rat was tested with U69,593 (0.48 mg/kg i.p.) or vehicle, 30 min before a nicotine (0.4 mg/kg s.c.) or a vehicle challenge and the start of locomotor recording. The sequence of tests was randomised for individual rats. On weekends following tests, rats were injected with 0.4 mg/kg nicotine s.c. and were left in their home cages.

#### *2.6. Statistics*

All data were analysed with one- or multiple-factor analysis of variance (ANOVA) for repeated measures as determined by the experimental design. The dependent variable was always the number of cage crosses, either the total scores for 60 min sessions or the results of individual periods of 10 min length. Post hoc Dunnett's tests were performed to compare activity scores after individual doses of each  $\kappa$ -agonist to vehicle under each nicotine condition. The maximum value for effects to be considered as significant was defined as  $P=0.05$ .

### 3. Results

#### 3.1. Development of nicotine sensitisation

An increase in activity scores over consecutive locomotor recordings started to show from the second nicotine-challenged session. Sensitisation progressed over three weeks, after which it reached a plateau (data not shown).

#### 3.2. Effects of $\kappa$ -agonists on nicotine-induced locomotor stimulation

As Fig. 1 demonstrates, graded doses of nicotine produced acute dose-dependent increases in total cage crosses in each of the three groups. Pretreatment with U50,488, U69,593 or CI-977 dose-dependently reduced this stimulation, with the highest doses blocking it completely.

A two-way repeated-measures ANOVA performed on data (total cage crosses) from the first of the two series of tests performed with U50,488 (examining doses of 0.5 and 1.5 mg/kg) revealed significant main effects of nicotine dose [ $F(3, 33)=46.8$ ,  $P<0.001$ ] as well as U50,488 dose [ $F(2, 22)=3.46$ ,  $P<0.05$ ], and a significant U50,488 $\times$ nicotine interaction [ $F(6, 66)=2.66$ ,  $P<0.05$ ]. In the second sequence of tests with U50,488 (3 mg/kg) against the same dose range of nicotine, main effects for nicotine [ $F(3, 33)=56.9$ ,  $P<0.001$ ] and U50,488 [ $F(1, 11)=37.3$ ,  $P<0.001$ ] were highly significant and so was the U50,488 $\times$ nicotine interaction [ $F(3, 33)=22.7$ ,  $P<0.001$ ]. In the group of rats tested with U69,593, the same analysis performed on total cage crosses revealed highly significant nicotine [ $F(3, 33)=31.8$ ,  $P<0.001$ ] and U69,593 [ $F(3, 33)=9.59$ ,  $P<0.001$ ] main effects as well as U69,593 $\times$ nicotine interaction [ $F(9, 99)=4.70$ ,  $P<0.001$ ]. In the third group tested with CI-977, main effects [for nicotine:  $F(3, 33)=106.0$ ,  $P<0.001$ ; for CI-

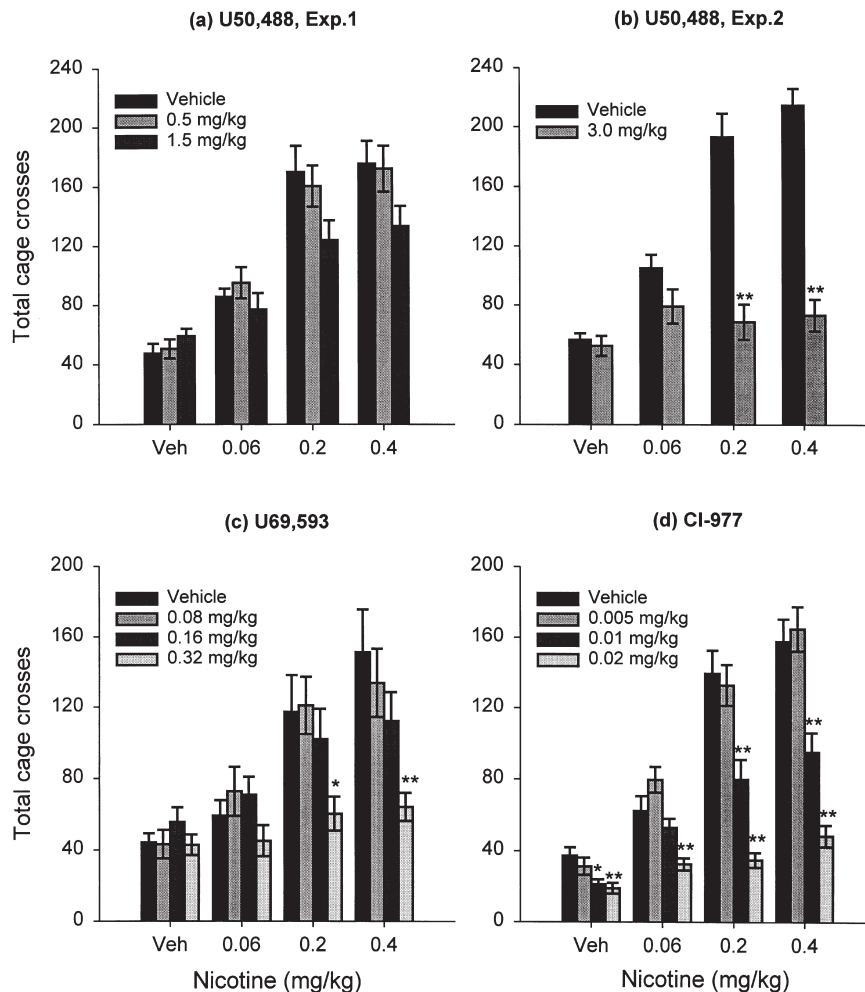


Fig. 1. The  $\kappa$ -opioid receptor agonists U50,488 (a and b), U69,593 (c) and CI-977 (d) dose-dependently reverse the locomotor stimulation induced by graded doses of nicotine in rats ( $n=12$  for each  $\kappa$ -agonist). In the absence of nicotine, U50,488 and U69,593 did not alter basal levels of motor activity, whereas CI-977 depressed it. Bars represent mean $\pm$ standard error of the mean (SEM) of the total number of cage crosses performed in 1 h sessions. Doses of each  $\kappa$ -agonist that produced significant differences compared with the vehicle under the respective nicotine condition are marked (\* indicates  $P<0.05$ , \*\* indicates  $P<0.01$ ; Dunnett's test).



977:  $F(3,33)=54.3$ ,  $P<0.001$ ] and the CI-977×nicotine interaction [ $F(9,99)=13.2$ ,  $P<0.001$ ] were equally strong.

Fig. 1 suggests that the interactions between nicotine and the  $\kappa$ -agonists arose from the fact that the  $\kappa$ -agonists reverse nicotine-induced increases in cage crosses while leaving basal activity levels unaffected. In fact, the total number of cage crosses following a saline challenge was not altered by U50,488 or U69,593, as confirmed by one-way repeated-measure ANOVA [for U50,488:  $F(2,22)=1.02$ , not significant (n.s.); for U69,593:  $F(3,33)=1.03$ , n.s.]. By contrast, CI-977 significantly reduced baseline levels of activity [ $F(3,33)=6.39$ ,  $P<0.01$ ] in a dose-related manner. Thus, whereas U50,488 and U69,593 modulated nicotine-induced motor stimulation without showing an effect in the absence of nicotine, CI-977 did not display this selectivity and caused locomotor depression when administered alone.

Fig. 2 demonstrates the time course for the effects of the largest dose of nicotine and of each  $\kappa$ -agonist, and for their interaction. Two-way repeated-measures ANOVA with pretreatment ( $\kappa$ -agonist or vehicle) and time period as factors were performed on these data. There was a significant main effect of time period in the experiments with U50,488 [ $F(5,55)=16.2$ ,  $P<0.001$ ], U69,593 [ $F(5,55)=41.3$ ,  $P<0.001$ ] and CI-977 [ $F(5,55)=36.7$ ,  $P<0.001$ ]; this effect reflects the overall higher activity in the first time period. Also, a significant interaction between time period and U69,593 was revealed [ $F(5,55)=3.23$ ,  $P<0.05$ ]; this may be explained by the absence of an effect of U69,593 in the first period. No such interaction was seen with U50,488 [ $F(5,55)=1.72$ , n.s.] or CI-977 [ $F(5,55)=1.49$ , n.s.]. In the absence of nicotine, motor activity after U50,488 or U69,593 almost paralleled baseline activity over time. CI-977 appears to depress basal activity in the first time

period only, which probably underlies its depressant effect on the total number of cage crosses in the absence of nicotine.

### 3.3. Effects of norbinaltorphimine

An initial three-way ANOVA was carried out with nor-BNI as a between-group factor and test condition (drug administered at the time of testing; i.e., U69,593/nicotine, U69,593/vehicle, vehicle/nicotine or vehicle/vehicle) and time period as within-group factors. The overall effect of nor-BNI was not significant [ $F(1,16)=2.18$ , n.s.], but a strong interaction of nor-BNI with test condition was found [ $F(3,368)=9.77$ ,  $P<0.001$ ]. In the light of this finding, separate analyses of variance were calculated to examine the interaction of nor-BNI with U69,593 in the presence and in the absence of nicotine.

For the nicotine condition, a three-way ANOVA with nor-BNI as between-group and U69,593 and time period as within-group factors revealed significant main effects of U69,593 [ $F(1,176)=5.67$ ,  $P<0.05$ ] and time period [ $F(5,176)=18.2$ ,  $P<0.001$ ]. No nor-BNI main effect was found [ $F(1,16)=2.96$ , n.s.]; however, nor-BNI interacted significantly with U69,593 [ $F(1,176)=6.87$ ,  $P<0.01$ ]. Fig. 3(a) demonstrates that U69,593 profoundly reduced nicotine-induced activity in the absence of nor-BNI, whereas the U69,593×nor-BNI interaction may be attributed to the blockade of this effect by nor-BNI. The main effect of time period reflects the general decline in activity over the first four periods. None of the interactions including time as a factor reached significance.

Fig. 3(b) visualises the effects of U69,593 and nor-BNI over time in sessions without nicotine. The marked overall decline in activity from the first to the second 10 min period is reflected by a highly significant main effect for time period [ $F(5,176)=43.6$ ,  $P<0.001$ ]. Neither

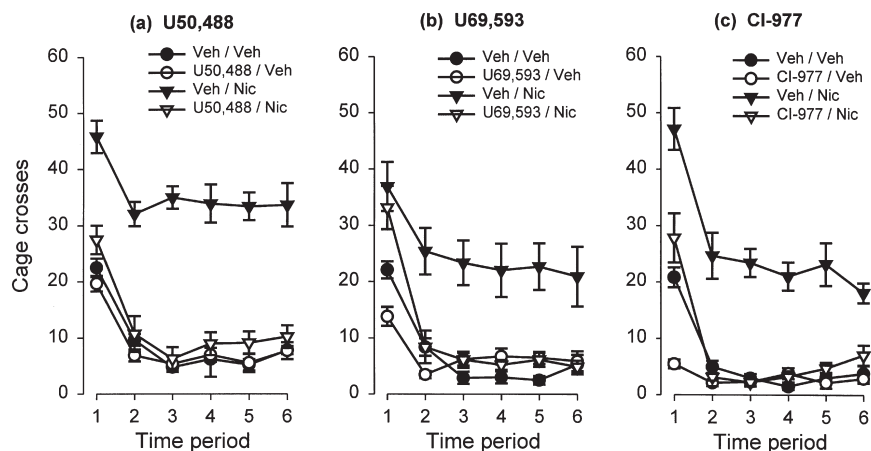


Fig. 2. The time course of effects of the highest tested dose of U50,488 (3.0 mg/kg), U69,593 (0.32 mg/kg) and CI-977 (0.02 mg/kg) on the locomotor stimulant effect of 0.4 mg/kg nicotine is shown. Each point in these and subsequent figures represents the mean±SEM of the number of cage crosses rats made in each 10 min interval of 1 h test sessions.

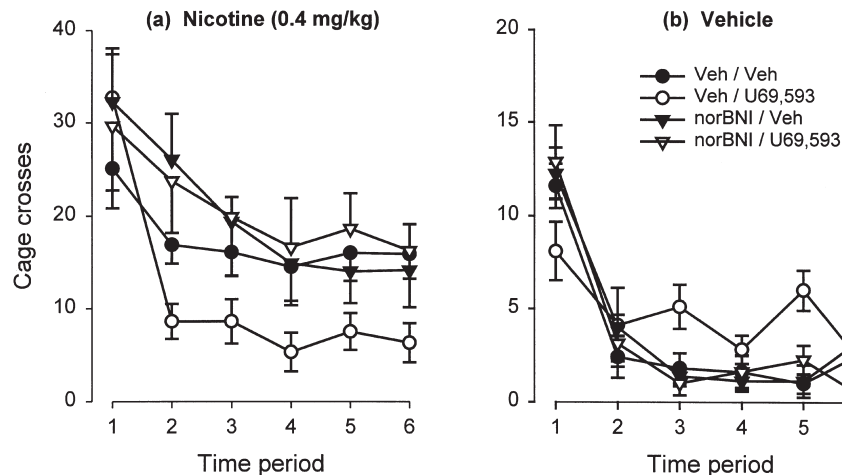


Fig. 3. The effects of U69,593 (4.8 mg/kg) on locomotor activity following (a) a nicotine challenge (0.4 mg/kg) or (b) a vehicle challenge are abolished in rats pretreated with the selective  $\kappa$ -receptor antagonist nor-BNI. 30  $\mu$ g nor-BNI ( $n=8$ ) or vehicle ( $n=10$ ) was administered i.c.v. to separate groups of rats on the day preceding tests. Note the different axes scales on the two graphs.

U69,593 [ $F(1, 176)=1.17$ , n.s.] nor nor-BNI [ $F(1, 16)=0.33$ , n.s.] had significant main effects, but both interacted with time [U69,593 $\times$ time:  $F(1, 176)=3.42$ ,  $P<0.01$ ; nor-BNI $\times$ time:  $F(1, 176)=2.73$ ,  $P<0.05$ ]. Both interactions probably result from the slight locomotor stimulation occurring after U69,593 in the third and fifth periods in the vehicle group, since this is the only test condition showing a difference to all other conditions. Neither the U69,593 $\times$ nor-BNI nor the U69,593 $\times$ nor-BNI $\times$ time interaction reached significance.

In order to examine if nor-BNI had any effect on either basal or nicotine-induced activity in the absence of U69,593, another three-way ANOVA with nor-BNI as between-group factor and nicotine and time as within-group factors was calculated on all data obtained after the vehicle for U69,593 was administered. In this analysis, neither the main effect of nor-BNI nor its interactions with nicotine or time period was significant.

#### 4. Discussion

The present experiments were conducted in rats that had been chronically pre-exposed to nicotine and displayed sensitisation to its locomotor-stimulant effects. In these animals, graded doses of nicotine produced an acute dose-related locomotor activation as reported earlier (Clarke and Kumar, 1983). Kappa-opioid receptor agonists dose-dependently attenuated this stimulation by nicotine. The effect could be shown with three different  $\kappa$ -selective agonists in separate groups of animals, which makes mediation via a non-specific action of any one compound very unlikely. However, Oka et al. (1998) found indication for a direct modulation of neuronal nicotinic receptors by these compounds. The authors report an inhibition of the nicotine-induced current by dynorphin A and U50,488, which was unaffected by the selec-

tive  $\kappa$ -opioid receptor antagonist nor-BNI. In the present study, nor-BNI reversed the effects of one of the  $\kappa$ -agonists (U69,593) on nicotine-induced behaviour. This provides evidence that these effects, and probably those of the other  $\kappa$ -agonists, were mediated by  $\kappa$ -opioid receptors and not by an unspecific action such as a direct blockade of nicotinic receptors.

Neither U50,488 nor U69,593 alone altered basal levels of locomotor activity. Activity had to be elevated by nicotine for these compounds to show locomotor depression, suggesting that their effects were not just due to sedation and behavioural disruption. This selectivity was not shared by CI-977, which significantly decreased spontaneous motor activity in the absence of nicotine. This observation is evidence against the possibility that a floor effect prevented the other two compounds from reducing baseline activity.

The pharmacological basis for this difference between U50,488 and U69,593 on the one hand, and CI-977 on the other hand, is unclear. CI-977 was found to bind with high affinity to [ $^3$ H]-U69,593 labelled  $\kappa$ -sites, and its  $\kappa$ -selectivity was demonstrated in a range of in vitro and in vivo essays (Hunter et al., 1990). The same study also reported on a dose-dependent suppression of basal locomotion by CI-977 in mice, but similar effects were found with U50,488, which clearly deviates from our findings with this compound in rats. U69,593 was reported to produce an initial period of hypoactivity followed by rebound hyperactivity in mice; the latter may resemble the slight motor activation that we found in certain time periods with U69,593 alone in the nor-BNI experiment.

Previous investigations of the effects of U50,488 and U69,593 on basal locomotor activity in rats yielded equivocal results: Di Chiara and Imperato (1988a) observed reductions in ambulation that accompanied the depression in accumbal and caudal dopamine concentrations induced by systemically administered  $\kappa$ -agonists

that included U50,488 at concentrations utilised in the present study. However, in agreement with findings reported here, an absence of basal locomotor depression was reported by Crawford et al. (1995) for U50,488 and by Heidbreder et al. (1993) for U69,593, at doses equaling or exceeding those tested in the present study. The basis for this discrepancy is uncertain. A major methodological difference between experiments by Di Chiara and Imperato and the other two studies lies in the way locomotor activity was recorded. The latter used the interruption of photobeams. The former group videotaped behaviour, rated it for several behavioural items including ambulation, and expressed it as the percentage of time spent for each item during 20 min intervals. A behaviour that would have produced beam breaks in a photocell cage could therefore be scored as an item other than ambulation. Thus, it is possible that the contradictory results evolved from focusing on slightly different aspects of motor behaviour.

The neurochemical mechanism underlying the behavioural interaction between nicotine and  $\kappa$ -opioid agonists was not investigated by the present study. However, the depressant effect of  $\kappa$ -agonists on dopamine release in the nucleus accumbens is well established (Di Chiara and Imperato, 1988a; Spanagel et al., 1990), and there is substantial evidence that an enhanced dopamine transmission in the same area underlies the locomotor-stimulant effects of nicotine (e.g., Louis and Clarke, 1998). Therefore, the suggestion that an acute reversal of the nicotine-induced increase in accumbal dopamine by  $\kappa$ -opioid receptor agonists underlies the observed acute antagonism of locomotor stimulation does not seem unduly speculative. A similar neurochemical interaction has been suggested to underlie parallel findings with cocaine: acute cocaine-induced locomotor stimulation was decreased by the pretreatment with U50,488 (5 mg/kg s.c.) or U69,593 (0.16 mg/kg s.c.) in rats with or without repeated pre-exposure to the respective  $\kappa$ -agonist (Crawford et al., 1995; Heidbreder et al., 1993). In contrast, Heidbreder et al. (1995) report that similar doses of U50,488 and U69,593 modified acute cocaine-induced activity only after being administered repeatedly for three days, and not when given as a single injection before the test session.

The fact that, in the present experiments, nor-BNI did not affect locomotor activity in the absence of U69,593 may appear surprising in view of its dopamine-enhancing effects in the accumbens (Spanagel et al., 1992; Maisonneuve et al., 1994). It has to be borne in mind, however, that it was administered about 17 h before recording locomotor activity. Although the blockade of  $\kappa$ -receptors by nor-BNI is long-lasting (Horan et al., 1992; Jones and Holtzman, 1992; Spanagel et al., 1994), the action on dopamine release has been shown to wear off after about 1 h, with dopamine concentrations

returning to baseline after 2 h (Maisonneuve et al., 1994).

In summary, the present study has shown a clear antagonism of nicotine-induced locomotor stimulation by three different  $\kappa$ -opioid receptor agonists. This effect is mediated by  $\kappa$ -opioid receptors and, at least in the case of two of the three compounds tested, is not due to a non-specific behavioural disruption. The nucleus accumbens is suggested as the neuroanatomical site of interaction and a reversal of nicotine-stimulated dopamine release as the underlying neuropharmacological mechanism. The latter will need to be confirmed by neurochemical investigation. Dopaminergic neurotransmission in the nucleus accumbens is also involved in the reinforcing effects of nicotine (Corrigall et al., 1992). Although the heterogeneous nature of this structure makes the similarity between the neuronal events underlying both behaviours uncertain, the present results with locomotor behaviour may fuel hypotheses about a possible role of  $\kappa$ -opioid receptors in nicotine reinforcement. An examination of  $\kappa$ -opioid agonists against the effects of nicotine in behavioural paradigms based on its rewarding properties, such as nicotine self-administration or conditioned place preference, will allow conclusions concerning a modulation of nicotine dependence by these compounds.

## Acknowledgements

This research was supported by a grant from the Medical Research Council. We thank Upjohn and Parke-Davis for generously providing the U50,488, U69,593 and CI-977, and Dr Rainer Spanagel for supplying the nor-BNI and for his advice.

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