

# Pharmacological stimuli decreasing nucleus accumbens dopamine can act as positive reinforcers but have a low addictive potential

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## Abstract

Opioid peptides, through  $\mu$  and  $\delta$  receptors, play an important part in reward. In contrast, the role of  $\kappa$  receptors is more controversial. We examined the possible positive reinforcing effects of a selective  $\kappa$  agonist, RU 51599, by studying intravenous self-administration in the rat. The effect of RU 51599 on dopamine release in the nucleus accumbens was also studied, as opioids and dopamine seem to interact in the mediation of reward. The behavioural and dopaminergic effects of RU 51599 were compared with those of the  $\mu$  agonist heroin. Rats self-administered both RU 51599 (6.5, 20 and 60  $\mu\text{g}/\text{inj}$ ) and heroin (30  $\mu\text{g}/\text{inj}$ ) at low ratio requirement. When the ratio requirement, i.e. the number of responses necessary to receive one drug infusion, was increased, self-administration of RU 51599 rapidly extinguished, whereas self-administration of heroin was maintained. Intravenous infusion of RU 51599 (100, 200 and 400  $\mu\text{g}$ ) dose-dependently decreased (25, 30 and 40%, respectively) extracellular concentrations of dopamine, as measured by means of microdialysis in freely moving rats. In contrast, heroin increased accumbens dopamine (130% over baseline). These results indicate that  $\kappa$  receptors, similarly to  $\mu$  ones, can mediate positive reinforcing effects of opioid peptides. However, the strength of the reinforcement is very low for  $\kappa$  receptors. This suggests that changes in accumbens dopamine do not correlate with the capacity of a stimulus to induce reward or aversion. In contrast, a parallel seems to exist between an increase in accumbens dopamine and the drive to reach or obtain a positive reinforcer.

## Introduction

During the last 30 years, a large research effort has been devoted to the understanding of the neurobiological substrates of reward. Comprehension of the physiology of reward is important not only in order to understand the biological basis of adaptive behaviour but also the possible substrate of drugs abuse. This research has suggested that opioid peptides are important in the mediation of reward (for review see Woods & Winger, 1987a; Ramsey & Van Ree, 1992). In general, it is believed that an increase in opioid activity has rewarding or positive-reinforcing effects whereas, a decrease in the action of these neuropeptides has aversive effects (Stolerman *et al.*, 1978; Mucha & Iversen, 1984; Almaric *et al.*, 1987; Hand *et al.*, 1988; for review see Iversen, 1983; Ramsey & Van Ree, 1992). Opioid peptides seem to interact with the mesolimbic dopaminergic (DA) system in the modulation of reward, and at least some of these effects of opioids are mediated by an activation of DA neurons (Phillips & Le Piane, 1980; Bozarth & Wise, 1981; Spyraiki *et al.*, 1983; Cooper, 1991; Shippenberg *et al.*, 1993; Altman *et al.*, 1996).

Different types of opioid receptors are expressed in the central nervous system (Lord *et al.*, 1976; Mansour *et al.*, 1987). Several

observations suggest that  $\delta$  and especially  $\mu$  opioid receptors are the substrate of rewarding effects of opioids (for review see Woods & Winger, 1987a). However, the role of  $\kappa$  receptors is less clear. Selective agonists of  $\kappa$  receptors have failed to show positive reinforcing effects (Lahti & Collins, 1982; Tang & Collins, 1985; Woods & Winger, 1987b) and even have aversive properties in certain behavioural tests (Mucha & Herz, 1985; Pfeiffer *et al.*, 1986; Shippenberg & Herz, 1987). Furthermore,  $\kappa$  agonists decrease dopamine release in the nucleus accumbens (Di Chiara & Imperato, 1988; Spanagel *et al.*, 1990), the DA projection area most involved in reward (Fibiger & Phillips, 1988; Wise & Rompré, 1989). In contrast, dynorphins the endogenous opioids that have the highest selectivity for  $\kappa$  receptors (Chavkin *et al.*, 1982; Corbett *et al.*, 1982; Schulz *et al.*, 1982) have, like the other opioid peptides, positive reinforcing effects (Khazan *et al.*, 1983; Iwamoto, 1988; Stevens *et al.*, 1991). Furthermore, selective  $\kappa$  agonists seem to increase the reinforcing properties of other opioid and DA drugs. Thus, their administration induces a shift towards the left of cocaine and heroin self-administration (Glick *et al.*, 1995).

In this report we have extended the study of the role of  $\kappa$  receptors in reward by studying possible positive reinforcing effects of a new  $\kappa$  agonist, RU 51599 (Hamon *et al.*, 1996) by means of intravenous self-administration (SA). These experiments were designed using drug-naïve animals and a SA schedule that was characterized by low ratio requirement and by the reinforcement of a response (nose-pokes) that is spontaneously provided at high rates by rodents. This procedure seems very sensitive for detecting positive-reinforcing effects of pharmacological compounds. For example, positive reinforcing effects of amphetamine were revealed within the first session of testing (Piazza *et al.*, 1989). This seems an important methodological point in the case of  $\kappa$  agonists. Indeed, positive reinforcing effects of these compounds have only been studied using either animals previously trained with other drugs or in complex self-administration procedures (for example see Lahti & Collins, 1982; Tang & Collins, 1985; Woods & Winger, 1987b) that might have masked the proper weak reinforcing effects of  $\kappa$  agonists. The effects of RU 51599 on DA release in the nucleus accumbens were also studied by means of microdialysis in freely moving animals. Accumbens DA was studied as this DA projection site seems the most involved in opioid-mediated reward. The DA and behavioural effects of RU 51599 were compared with those of the  $\mu$  agonist heroin.

## Materials and methods

### General methods

#### Subjects

Male Sprague–Dawley rats (Iffa Credo; Lyon, France) weighing 300–320 g were used. Animals were individually housed under a 12 : 12 h dark/light cycle (lights off at 12 a.m.) and with constant temperature (22 °C) and humidity (66%). Animals had *ad libitum* access to food and water upon arrival. After at least 1 week of acclimatization to the animal facilities, their body weight was progressively brought to 90% of their initial weight by decreasing the daily ration of food. Food was given at 16.00 h, and animals were food restricted for at least 12 days before the start of experiments, then and maintained for the duration of the studies.

#### Constitution of experimental groups

As it has been shown that locomotor response to novelty is correlated to the reinforcing and DA effects of drugs (Piazza *et al.*, 1989; Hooks *et al.*, 1991; Rougé-Pont *et al.*, 1993), we ensured a homogeneous distribution of this factor throughout the different experimental groups. For this purpose, on the morning prior to the start of the food restriction procedure, animals were tested for their response to novelty. Locomotor activity was tested in circular corridors (10 cm wide and 70 cm in diameter) between 08.00 and 10.00 h by means of four photoelectric cells placed at the perpendicular axis of the apparatus. Animals were then evenly distributed among the different groups according to their score accumulated over the 2 h of testing.

#### Drugs

All drugs for intravenous administration were freshly prepared in sterile 0.9% NaCl solution. RU 51599 (*trans-N*-2,3,-dihydro 2(1-pyrrolidinyl)1H-inden-1-yl/*n*-methyl-3-nitrobenzene acetamide hydrochloride) was kindly donated by Roussel Uclaf (Romanville, France) and doses expressed as RU 51599 base. Heroin HCl (Francopia, Gentilly, France) was used and doses were expressed as the salt of the drug.

#### Stereotaxic surgery

Animals undergoing stereotaxic surgery for the implantation of a microdialysis guide cannula were anaesthetized with sodium pentobarbital (50 mg/kg, *i.p.*) and placed in a stereotaxic apparatus (Kopf Instruments, CA, USA) with the incisor bar 5.0 mm above the interaural line. The chronic guide cannula (CMA/11, Carnegie Medicine Sweden) was implanted above the nucleus accumbens and lowered 2 mm above the location of the probe tip, at an angle of + 6° using the following coordinates: AP + 3.5, L + 1.9, V – 6.5 relative to the bregma and surface of the skull (Pellegrino *et al.*, 1979). The guide cannula was secured in place with the use of stainless steel screws and dental cement, and its removable stainless steel stylet was left in place to prevent clogging. Animals were then left to recover for at least 1 week before undergoing surgery for the implantation of the intravenous catheter.

#### Implantation of catheters for intravenous administration

For intravenous drug administration, intracardiac silastic catheters (Dow Corning, Midland, MI, USA) were implanted under ether anaesthesia. The catheter (20  $\mu$ L dead volume) was inserted in the right auricle through the external jugular vein, passed under the skin and fixed in the mid-scapular region. Animals were allowed to recover at least 1 week before the start of either the SA or the microdialysis experiment.

#### Intravenous self-administration

Daily sessions were performed for 1 h during the dark period (13.00 h). At the start of each session, the external end of the catheter was connected to a pump-driven syringe and the catheter filled with the drug to be self-administered. The SA cage (35  $\times$  35 cm floor area, 50 cm high, Imetronics, Bordeaux, France) had two holes placed symmetrically in the centre of two opposite walls. When the animal introduced its nose (nose-poke) in one of the holes (defined as active), it switched on the pump which delivered a 20  $\mu$ L injection (*inj*) of a given solution for 2 s. Subsequent nose-pokes during this period had no effects on the injection pump, but were recorded. Nose-pokes in the other hole (defined as inactive) had no effects at any time. The number of nose-pokes in both holes and the number of injections were recorded throughout the experiments. At the end of each session, the catheter was filled with 100  $\mu$ L of a heparin–streptokinase solution (10 and 3000 IU, respectively) to prevent clogging.

#### Microdialysis

Two days before the microdialysis test, a dialysis probe (CMA/11, 2 mm cuprophane membrane length, Carnegie Medicine, Stockholm, Sweden) was inserted through the guide cannula, and animals returned to their home cage. For each probe, the *in vitro* recovery had been determined before its implantation in order to homogenize this factor throughout the groups. On the day of testing, each animal was transferred to the dialysis cage (32  $\times$  32  $\times$  22 cm), the probe was connected to a pump-driven (Harvard 22, Harvard Apparatus, South Natick, MA, USA) syringe via a two-channel swivel, and the perfusion started immediately at a flow rate of 2  $\mu$ L/min. The perfusion fluid was a modified artificial cerebrospinal fluid containing (in mM): NaCl, 145; CaCl<sub>2</sub>, 1.2; KCl, 2.7; MgCl<sub>2</sub>, 1.0; Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub>, 0.2 (buffer) at pH 7.4. Dialysate samples were collected in 40  $\mu$ L sample loops and injected with a fully automated on-line system (Rheodyne 7125 in combination with a sample/event controller Touzart *et al.* Matignon, Cowtaboeuf, France) into the HPLC system consisting of a Shimadzu LC-9A pump and an analytical column (Hypersil BDS-

C18, 3  $\mu\text{m}$ , 100  $\times$  4.6 mm, Touzart et Matignon, France). The mobile phase was delivered at a constant rate of 0.9 mL/min, and consisted of a sodium phosphate buffer (75 mM) containing 20  $\mu\text{M}$  of EDTA, 1.5 mM of sodium dodecyl sulphate, 100  $\mu\text{L/L}$  of triethylamine, 15% methanol and 13% of acetonitrile, pH 5.6. A model 5020 guard cell was positioned before the automatic injector to oxidize at +175 mV. A 5011 Analytical Cell was placed after the column. The first electrode oxidized at +350 mV and the second reduced at -260 mV to quantify only dopamine. A coulometric detector (Coulochem II, ESA, Bedford, MA, USA) was used to detect DA. Signals were recorded with a D2000 Mega integrator (Merck, Nogent-sur-Maine, France). The retention time of dopamine was 7.5 min and the detection limit of this compound during the assay was of 0.5 pg/40  $\mu\text{L}$ . At 1–6 days from the end of the experiment, rats were anaesthetized with sodium pentobarbital and perfused transcardially with 50 mL of 0.9% NaCl solution followed by 200 mL of 4% formaldehyde solution. Brains were removed and the precise location of the cannula determined 100  $\mu\text{m}$  thionine-stained coronal sections. Only animals with correctly placed probes were included in the statistical analyses.

## Procedures

### Acquisition of intravenous self-administration

Four different groups of animals were allowed to self-administer one of three different doses of RU 51599 (6.5  $\mu\text{g}/\text{inj}$ ,  $n = 7$ ; 20  $\mu\text{g}/\text{inj}$ ,  $n = 8$ ; or 60  $\mu\text{g}/\text{inj}$ ,  $n = 8$ ) or heroin (30  $\mu\text{g}/\text{inj}$ ,  $n = 6$ ) over a period of 5 days. A fixed ratio (FR) of 1 (FR1) was used for this study, i.e. the animals had to do one nose-poke to obtain one injection. In this case, the comparison of the number of nose-pokes between the active and inactive holes was used to attest self-administration. A given group was considered to develop self-administration when the number of nose-pokes in the active hole was significantly higher than the number of nose-pokes in the inactive one.

### Self-administration under an intersession progressive ratio schedule

This phase started after the acquisition phase and lasted 9 days. Over this period of time, the ratio (number of nose-pokes) required to obtain one injection was progressively increased from sessions one to eight. The following schedule was used: FR3 (3 days), FR5 (4 days) and FR8 (2 days). In this phase of the test, the modifications of the number of nose-pokes in the active hole and injections were used to attest the strength of drug reinforcement, the prediction being that if a drug has strong reinforcing properties, then SA behaviour is maintained constant even when the ratio to obtain the drug is increased. Owing to catheter failure, the number of animals that remained in this study was  $n = 5$ , 6 and 7 for the RU 51599 groups (6.5, 20 and 60  $\mu\text{g}/\text{inj}$ , respectively), and  $n = 5$  for heroin.

### Extracellular concentrations of dopamine in the nucleus accumbens

On the day of the microdialysis experiment, animals were placed in the dialysis cage at 12 p.m. and perfusion started immediately. Following at least three consecutive dialysate samples which varied for less than 10% between each other (baseline value), the animals received an intravenous injection of one of three doses of RU 51599: 100  $\mu\text{g}$  ( $n = 4$ ), 200  $\mu\text{g}$  ( $n = 5$ ), 400  $\mu\text{g}$  ( $n = 5$ ) or of heroin 90  $\mu\text{g}$  ( $n = 5$ ) or saline ( $n = 4$ ), in a 100  $\mu\text{L}$  volume. Extracellular concentrations of DA were monitored for 3 subsequent hours. The low doses of RU 51599 was chosen because it corresponded to the mean of the loading dose (five injections) observed during self-administration of the medium dose (20  $\mu\text{g}/\text{inj}$ ) of this compound. Similarly, the dose of heroin corresponded to the mean of the loading dose for this drug

(three injections). The loading dose is the quantity of drug that is rapidly self-administered at the beginning of the SA session. The first 5 min of the session were used to calculate the loading dose.

## Statistical analyses

Data were analysed with analysis of variance (ANOVA) for repeated measures. For the analysis of SA, the dose of the drug was considered a between-group factor, and the hole (active or inactive) as the first within-group factor and the different days of testing as the second within-group factor. Basal levels of DA (in pg/40  $\mu\text{L}$ ) were analysed considering the dose as a between-group factor and the mean of the last three samples before the intravenous injection as the dependent variable. Drug-induced changes in DA were analysed as a percentage of baseline values. Dose was the between-group factor and the time post-injection the within-group factor. *Post hoc* tests (Newman-Keuls) were used where appropriate.

## Results

### Acquisition of intravenous self-administration

Figure 1 shows that the  $\kappa$  agonist RU 51599 induced self-administration (hole effect,  $F_{1,20} = 27.47$ ,  $P < 0.001$ ) and that this effect was dependent on the dose used (hole  $\times$  dose interaction,  $F_{2,20} = 3.62$ ,  $P < 0.04$ ). Over the 5 days of testing, the number of nose-pokes in the active hole was significantly higher than the number of nose-pokes in the inactive one for all doses tested (hole effect,  $F_{1,6} = 25.66$ ,  $P < 0.002$ ;  $F_{1,7} = 13.38$ ,  $P < 0.008$  and  $F_{1,7} = 9.00$ ,  $P < 0.02$  for 6.5, 20 and 60  $\mu\text{g}/\text{inj}$ , respectively). Furthermore, the dose-response curve for RU 51599 SA presented an inverted U shape, i.e. the number of injections increased between the low (6.5  $\mu\text{g}/\text{inj}$ ) and the medium dose (20  $\mu\text{g}/\text{inj}$ ) and diminished when the dose was further increased (60  $\mu\text{g}/\text{inj}$ ). Such a shape, typical of SA experiments, is clearly shown in Fig. 2 that represents the mean of the number of injections over the last 3 days of testing for each dose. In our experimental conditions, heroin also induced acquisition of SA as shown by the higher number of nose-pokes in the active hole compared with the inactive one (hole effect  $F_{1,5} = 41.73$ ,  $P < 0.001$ ).

### Self-administration under an intersession progressive ratio schedule

When the ratio to obtain the drug was increased, all animals self-administering RU 51599 showed a progressive decrease in SA behaviour (Fig. 3). Thus, the number of nose-pokes in the active hole and the number of injections decreased throughout the sessions (days effect, nose-pokes:  $F_{13,221} = 5.10$ ,  $P < 0.001$ ; injections:  $F_{13,221} = 13.65$ ,  $P < 0.001$ ) and this for all the doses tested. The extinction of SA under the progressive ratio schedule was directly related to the dose of RU 51599 (Fig. 4). Thus, the higher the dose of RU 51599 used, the greater the percentage decrease in responding over the progressive ratio schedule (60  $\mu\text{g}/\text{inj}$  vs. 6.5  $\mu\text{g}/\text{inj}$ ,  $P < 0.05$ ). A decrease in SA behaviour in response to the increase in ratio was specific to the  $\kappa$  agonist. In fact, for heroin opposite results were observed (Fig. 3). Thus, the number of nose-pokes increased with the increase in ratio (days effect,  $F_{13,52} = 5.18$ ,  $P < 0.001$ ), whereas the number of self-injections remained fairly constant.

### Extracellular concentrations of dopamine in the nucleus accumbens

Baseline values (in pg/40  $\mu\text{L}$ ) did not differ between the five experimental groups (group effect,  $F_{4,48} = 0.07$ ,  $P > 0.98$ ) and their mean was  $4.3 \pm 0.8$  pg/40  $\mu\text{L}$ . Comparison of the percentage changes in

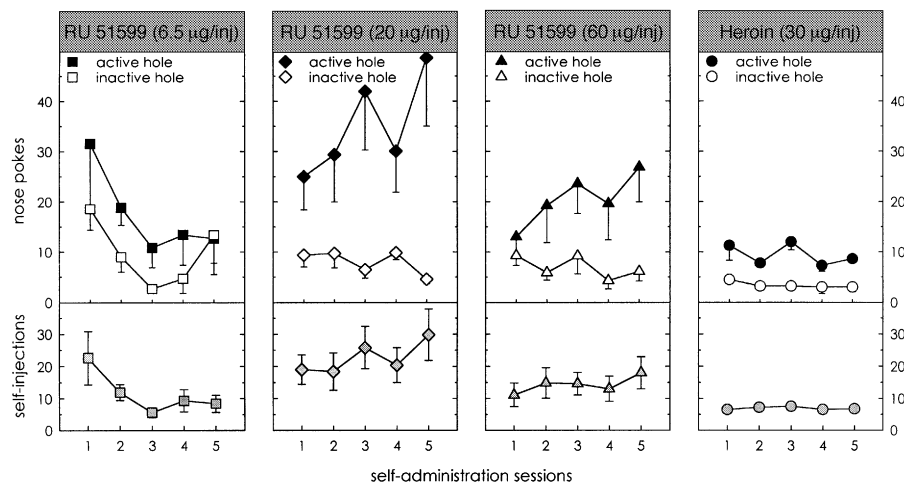


FIG. 1. Self-administration of RU 51599 (6.5, 20 and 60 µg/inj) and heroin (30 µg/inj) during the acquisition phase. All animals carried out SA, thus (top panels), the number of nose-pokes in the active hole was significantly higher than the number of nose-pokes in the inactive one. The number of self-injections for each dose of RU 51599 tested and for heroin are shown in the bottom panels.

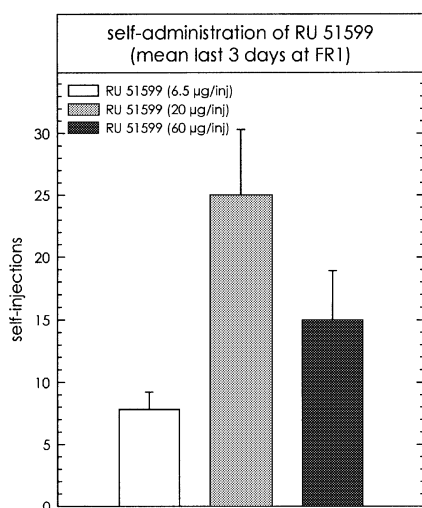


FIG. 2. Dose-dependent self-administration of RU 51599 during the acquisition phase. The mean number of self-injections (averaged over the last 3 days of the acquisition phase) presents an inverted U shape. Thus, the number of self-injections increased between the low (6.5 µg) and the medium dose (20 µg) and diminished when the dose was further increased (60 µg).

DA after RU 51599 and saline injections revealed a significant overall difference between these groups [treatment effect,  $F_{3,14} = 44.00$ ,  $P < 0.001$ ], *post hoc* analysis showing that all the doses of RU 51599 were significantly different from saline (saline vs. 100, 200 and 400 µg of RU 51599,  $P < 0.001$  in all cases). Thus, an analysis including the three RU 51599 groups and comparing percentage changes in DA over the three time points before and after the injection indicated a significant decrease in DA after RU 51599 (injection effect,  $F_{1,11} = 206.7$ ,  $P < 0.001$ ). In contrast, a similar analysis performed on the results obtained after the saline injection indicated a slight but significant increase in DA after this treatment (injection effect,  $F_{1,3} = 84.11$ ,  $P < 0.01$ ) (Fig. 5, left panel). The comparison of the percentage change in DA after the injection of RU 51599 indicated that the decrease in DA induced by this drug was dose dependent (dose effect,  $F_{2,11} = 4.08$ ,  $P < 0.05$ ), as revealed by a *post hoc* analysis, the effects of the lower dose of RU 51599 were significantly different from those of the highest one ( $P < 0.05$ ). During the first hour in which the maximal effect of the drugs are

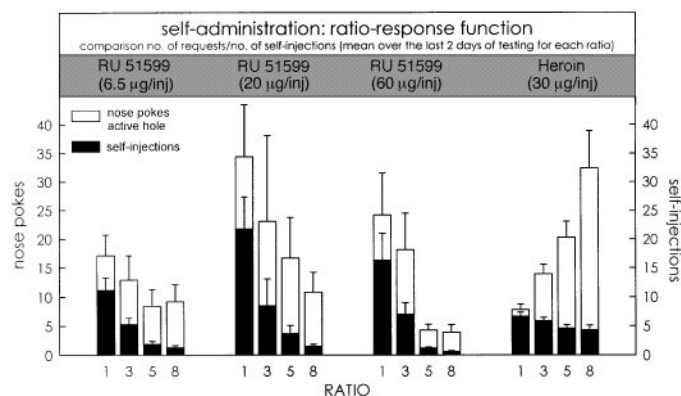


FIG. 3. Self-administration of RU 51599 (6.5, 20 and 60 µg/inj) and heroin (30 µg/inj) during the intersession progressive ratio schedule. The number of nose-pokes is matched to the number of self-injections for each ratio requirement (mean over the last 2 days of testing for each ratio). The increase in ratio produced a progressive decrease in SA behaviour for animals self-administering RU 51599, whereas this behaviour remained stable for animals self-administering heroin. Thus, the number of nose-pokes and self-injections decreased for all the RU 51599 groups in a ratio-dependent manner; in contrast, the number of nose-pokes increased in parallel to the increase in ratio for the heroin group to maintain a stable drug intake.

observed, the mean decrease in DA levels was 25, 30, and 40% for doses 100, 200 and 400 µg, respectively (Fig. 5, central panel).

The effects of heroin were opposite to those of RU 51599. The comparison of the percentage change in DA before and after the injection of heroin (the first three time point in each case) indicated a significant increase in DA concentration after this drug (injection effect,  $F_{1,4} = 317.87$ ,  $P < 0.001$ ). Heroin-induced increase in DA was significantly higher than the one induced by the saline injection, as revealed by the between-group comparison of the percentage change in DA after the two injections (treatment effect,  $F_{1,7} = 194.09$ ,  $P < 0.001$ ). Over the first hour, the mean increase over baseline was about 130% for heroin and 15% for saline (Fig. 5, right panel).

## Discussion

Our results show that both  $\kappa$  (RU51599) and  $\mu$  (heroin) agonists can have positive reinforcing effects in the context of an SA test.  $\kappa$  and  $\mu$  agonists, however, differed in the strength of reinforcement. Indeed,

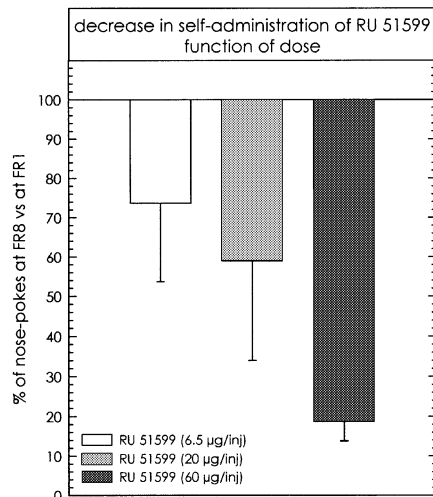


FIG. 4. Variation in SA of RU 51599 under the progressive ratio schedule. The decrease (percentage of number of responses in the active hole at FR8 with respect to the number of responses at FR1) was directly related to the dose of RU 51599. Thus, the higher the dose, the greater the decrease in responding.

when the work necessary to obtain one injection was increased, SA was maintained only for heroin. Finally, the two compounds also profoundly differ in their DA effects, as RU51599 decreased extracellular concentrations of DA in the nucleus accumbens, whereas heroin increased them.

The findings of the present experiments show that selective  $\kappa$  agonists can have positive reinforcing effects. Thus, SA of RU 51599 in the acquisition phase had two important criteria which were used to define a drug as a positive reinforcer in this context. First, a higher number of nose-pokes in the active hole was observed, which shows that the stimulus is actually able to increase the probability of appearance of a response. Second, an inverted U-shaped dose-response function was observed, with a peak for the middle dose. This shape is typical of SA experiments: animals do not self-administer too low a dose that is not reinforcing enough, and try to maintain an optimum level of reinforcement by decreasing the responding when the dose is too high (for example, see Deminière *et al.*, 1989).

Despite such clear positive reinforcing effects, RU 51599 was revealed as a very weak reinforcer, as responding was not maintained once the ratio requirement to obtain the drug was increased. This observation suggests that, as for other  $\kappa$  agonists (Woods & Winger, 1987a; Ramsey & Van Ree, 1992), RU 51599 should be devoid of addictive properties. Indeed, for addictive drugs, such as heroin in the present experiment, an increase in ratio is followed by a parallel increase in responding. In this respect, studies using progressive ratio schedules have shown that animals can provide up to hundreds of responses to obtain a single injection of either cocaine or heroin (Roberts *et al.*, 1989; Roberts & Bennet, 1993).

This is the first time, to our knowledge, that a  $\kappa$  agonist has been shown to induce SA. Although Khazan *et al.* (1983) had reported that dynorphin maintains self-administration in rats trained to self-administer morphine, most studies report that  $\kappa$  agonists are not self-administered (Lahti & Collins, 1982; Tang & Collins, 1985; Woods & Winger, 1987b). It is unlikely that differences between our results and those of the literature are due to a non-selectivity of RU 51599. The selectivity for  $\kappa$  receptors of RU 51599 is comparable with the one of the classically used  $\kappa$  agonist, U 50488 (Hamon *et al.*, 1996).

Furthermore, as other  $\kappa$  agonists, RU 51599 decreases extracellular concentrations of DA and induces place aversion (M. Marinelli, A. Dekeyne, C. Oberlander, M. Le Moal, H. Simon, P.V. Piazza, unpublished observations). It is possible that the condition we used for the SA acquisition study may have explained the weak reinforcing effects of RU 51599. Indeed, in our paradigm, we reinforced a spontaneously highly expressed behaviour (nose-pokes); animals had no previous experience of operant training and a low schedule of reinforcement was used. In contrast, most studies on SA of  $\kappa$  agonists have used learned responses, induced by training animals with other drugs (Woods & Winger, 1987b), or high ratio schedules of reinforcement (Lahti & Collins, 1982; Tang & Collins, 1985). In those conditions reinforcing properties of  $\kappa$  agonists are probably not strong enough to be revealed.

The results of the acquisition phase also suggest that reward and aversion are not necessarily linked to parallel changes in accumbens DA. Indeed, an increase in accumbens DA has been previously associated with an increase in diverse pleasurable effects, whereas its decrease has been associated with dysphoria and anhedonia or with the reduction of the 'hedonic' value of psychoactive drugs, brain stimulation and palatable foods (Wise & Bozarth, 1985; Wise, 1985; Bozarth, 1991; Funada *et al.*, 1993). However, this does not seem to be the case, as RU 51599 dose-dependently reduced DA levels in the nucleus accumbens and, in spite of this, its positive reinforcing effects suggest that it can act as a rewarding stimulus.

The results of the progressive ratio schedule support the idea (for review see Robinson & Berridge, 1993; Berridge, 1996; Di Chiara, 1998), that DA plays a part in mediating the motivational drive that leads to the search for reinforcing stimuli. Thus, the propensity to work at obtaining the reinforcer was positively correlated to drug-induced changes in extracellular concentrations of accumbens DA. For heroin, which increased DA release in the nucleus accumbens, animals readily increased the amount of responses required by the progressive ratio schedule in order to maintain a constant level of SA. In contrast, for RU 51599, that dose-dependently decreased DA release, SA rapidly extinguished when the amount of work to obtain the drug was increased. The apparent direct relationship between the motivational drive for the reinforcer and accumbens DA is backed by the fact that during the progressive ratio schedule, the decrease in responding was directly related to the dose of RU 51599. That is, the higher the dose of self-administered RU 51599, and consequently probably the greater the decrease in DA release, the lower the work the animal was ready to provide to obtain an infusion of the drug.

It could be argued that the changes in DA observed during the microdialysis experiment, in which the drugs were non-contingently administered, and the ones actually taking place during SA are different. The only strongest evidence supporting this idea is the observation made by Hemby *et al.* (1995) that during heroin SA extracellular concentration of DA in the nucleus accumbens does not increase (Hemby *et al.*, 1995). In fact, other research groups have published opposite results and consistently found an increase in extracellular DA during heroin SA (Kiyatkin *et al.*, 1993; Wise *et al.*, 1995; Xi *et al.*, 1998). These discrepancies suggest that it is probably more the location of the microdialysis probe than the mode of infusion (contingent/non-contingent) which determine a change in DA levels. Thus, it has been recently shown that in response to the intravenous infusion of low doses of opioid drugs significant changes in DA are largely restricted to the shell of the nucleus accumbens (Pontieri *et al.*, 1995; Tanda *et al.*, 1997).

A selective implication of DA in mediating the motivational properties of reinforcing stimuli is confirmed by different experimental evidence (for review see Berridge, 1996). For example, a lesion of

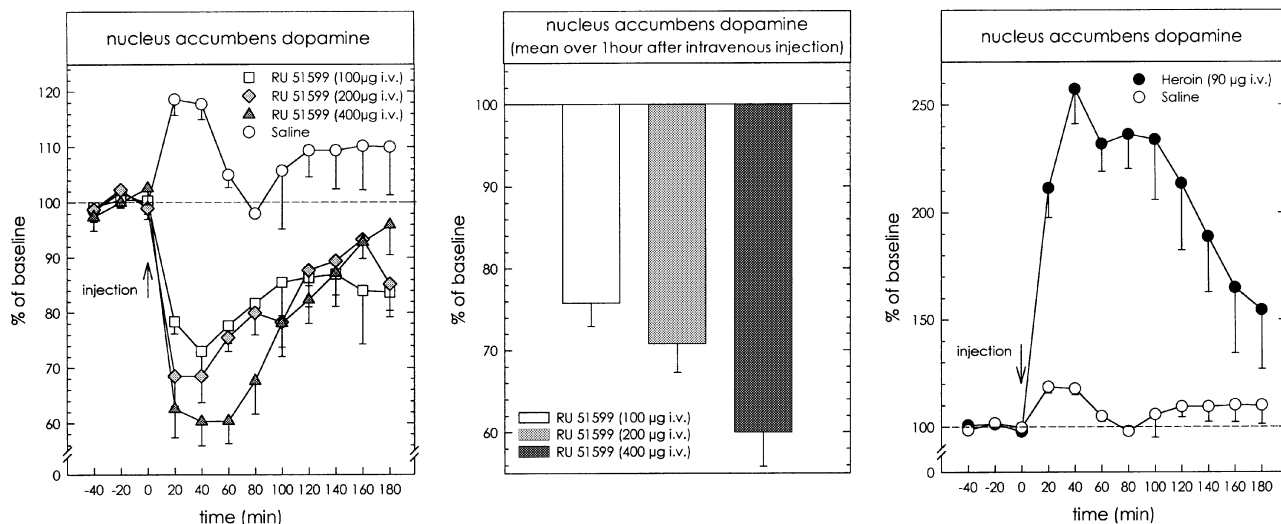


FIG. 5. Effects of the intravenous administration of RU 51599 (100, 200 and 400 µg), heroin (90 µg) or saline on extracellular concentrations of DA in the nucleus accumbens. Intravenous administration of RU 51599 induced a decrease in extracellular concentrations of DA compared with intravenous administration of saline (left panel). This decrease in DA was dose-dependent (central panel). Thus over the first hour, the mean decrease in accumbens DA was of 25, 30 and 40% for doses 100, 200 and 400 µg of RU 51599, respectively. In contrast to RU 51599, heroin induced a marked increase in accumbens DA (right panel).

mid-brain DA neurons does not modify the hedonic or aversive reactions of rats to sweet or bitter solutions, as measured with the taste reactivity test, which does not require approach behaviour (Berridge *et al.*, 1989), whereas it modifies the amount of palatable food taken (Salamone *et al.*, 1991; Cousins *et al.*, 1993). In parallel, lesion of mesencephalic DA neurons seems to have a higher impact on appetitive than on consumatory behaviours (for review see Robbins & Everitt, 1996). Appetitive behaviours include the behavioural sequences that lead to a reinforcer, whereas consumatory behaviours are those performed in its presence, e.g. eating or sexual mounting (Robbins & Everitt, 1996). Finally, using high-speed chronoamperometry it has been shown that DA-related oxidation current starts to increase just before the animals initiate responding (lever pressing) for food and further increases during the lever pressing. In contrast, a sharp decrease in the DA signal is observed once the earned food is retrieved (Kiyatkin & Gratton, 1994).

The behavioural and neurochemical distinction made in our experiments between positive reinforcing effects of a pharmacological stimulus and its strength to act as a positive reinforcer have consequences in the current theory of drug abuse. Traditional views consider the activation of the mesocorticolimbic DA system as a major substrate of drug-induced reward (for review see Fibiger & Phillips, 1988; Wise & Rompré, 1989; Bozarth, 1991). Our results suggest that this system participates in determining the strength of drug-reinforcement, which may reflect abuse potential. However, an increase in accumbens DA does not seem to be the necessary condition determining the positive reinforcing effects of a drug.

These observations prompt the hypothesis that positive reinforcing and addictive effects of drugs are two independent phenomena mediated by different neural substrates. An increase in DA could then specifically be one of the determinants of the addictive potential of a drug. This idea is in line with and extends recent findings and theoretical constructs of Di Chiara's group (Bassareo & Di Chiara, 1997; Di Chiara, 1998). These authors have shown that an increase in nucleus accumbens DA is a common response to the first exposure of very palatable food and drugs of abuse (Bassareo & Di Chiara, 1997). However, over repeated exposure only drugs of abuse keep increasing extracellular DA (Bassareo & Di Chiara, 1997). It is to

this non-habituating increase in extracellular concentration of DA that the addictive potential of drugs have been attributed (Di Chiara, 1998). Our results showing that positive reinforcing effects of a given compound are independent of an increase in DA but that the strength of its reinforcing effect is related to the release of this neurotransmitter complement and are in line with this hypothesis.

In conclusion, our results show that an increase in accumbens DA is not a necessary condition for a stimulus to act as a positive reinforcer. Indeed, even a stimulus that actually decreases DA can have positive reinforcing effects. In contrast, a parallel seems to exist between an increase in the release of DA and the motivation to reach or obtain a positive reinforcer.

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### Abbreviations

DA	dopamine
inj	injection
SA	intravenous self-administration

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