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Delta opioid discrimination learning in the rat Assessment with the selective delta agonist SNC80

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Abstract

The majority of reports assessing opioid drug discrimination learning (DDL) have concentrated on characterizing the stimulus properties of compounds selective for mu and kappa opioid receptors. Assessments of delta opioid DDL have been limited and, to date, these assessments have been restricted to the monkey and pigeon. No assessment of delta stimulus control has been examined in rodents. To that end, the present experiment examined discriminative control by the selective delta agonist SNC80 in rats and its generalization to and antagonism by compounds relatively selective to the delta and mu receptor subtypes using the conditioned taste aversion baseline of DDL. Animals injected with 5.6 mg/kg of SNC80 prior to a saccharin–LiCl pairing and with the SNC80 vehicle prior to saccharin alone acquired the discrimination within seven conditioning cycles. The discriminative effects of SNC80 were maximal at 20 min, partial at 120 min, and lost at 240 min. The discrimination was dose dependent in that as the dose of SNC80 increased, the amount of saccharin consumed decreased. In subsequent generalization tests, the delta agonist SNC162 produced SNC80-appropriate responding at a dose of 18 mg/kg. Conversely, the mu agonist morphine produced vehicle-appropriate responding at all doses tested. These selective generalization patterns with SNC162 and morphine suggest that the discriminative effects of SNC80 are mediated at the delta, but not the mu, receptor, a conclusion supported by the fact that SNC80's discriminative control was completely blocked by the delta-selective antagonist NTI, but not by the mu-selective antagonist naltrexone. The present findings indicate that not only do rats readily discriminate both mu- and kappa-selective agonists from their respective vehicles, but they also discriminate compounds that are selective for the delta receptor subtype, thus extending the class of compounds that can serve such discriminative functions for the rat.

Keywords: Delta opioid; SNC80; Naltrindole; Drug discrimination learning; Conditioned taste aversion; Rat

1. Introduction

Although the discriminative stimulus effects of a variety of opioid compounds have been well characterized (Bigelow and Preston, 1989; Dykstra et al., 1997), such assessments have been limited primarily to those compounds with relative selectivity for the mu (Bertalmio and Woods, 1987; France et al., 1984; Gianutsos and Lal, 1976; Grabus et al., 1999; Herling et al., 1984; Hill et al., 1971; Jarbe, 1978; Locke and Holtzman, 1986; Morgan and Picker, 1998; Schaefer and Holtzman, 1977; Shannon and Holtzman, 1976, 1977a,b; Smurthwaite and Riley, 1994; Stevenson et al., 1992; Suzuki et al., 1995; Ukai and Holtzman, 1988; Winter, 1975) and kappa (Negus et al., 1990, 1996; Picker, 1994; Picker and Dykstra, 1987, 1989; Picker et al., 1990, 1996; Pournaghash and Riley, 1993; Schaefer and Holtzman, 1978) subtypes of the opiate receptor. Work assessing the discriminative stimulus effects of delta agonists, on the other hand, has been quite limited (Brandt et al., 1999; Comer et al., 1993; Jewett et al., 1996; Negus et al., 1994, 1998; Picker and Cook, 1998).

In the first study assessing delta agonists as training drugs within the drug discrimination procedure (Comer et al., 1993), pigeons were trained to discriminate the systemically active, delta agonist BW373U86 (Chang et al., 1993) from sterile water in a two-key, food-reinforced procedure. Animals acquired the discrimination in approximately 100

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training sessions and were subsequently tested for the generalization of BW373U86 control to a variety of compounds with varying degrees of selectivity for mu, delta, and kappa receptor subtypes. BW373U86 partially generalized to several other systemically active, delta agonists, e.g., oxymorphindole and LY123502, but failed to generalize to either the delta peptides DPDPE or DSLET (intracerebroventricular administration). BW373U86 also generalized partially to morphine, alfentanil, and ethylketocyclazocine. These generalization patterns are consistent with the general low selectivity of BW373U86 for the mu and delta receptor subtypes (Negus et al., 1996; Picker and Cook, 1998). Although nonselective, BW373U86's stimulus effects were fully blocked by the delta antagonist naltrindole at doses 1000-fold less than those needed to block morphine's stimulus effects, an effect suggestive of delta mediation of its discriminative properties.

Subsequent to these findings, Jewett et al. (1996) trained pigeons to discriminate the delta agonist DPDPE (intracerebroventricular administration) from saline in a two-key, food-reinforced procedure. Animals acquired the discrimination relatively rapidly and subsequently generalized DPDPE stimulus control to the highly selective peptidergic delta agonists, DSLET and deltorphin II (but not to the mu-selective peptide, DAMGO). Interestingly, BW373U86 substituted only partially for DPDPE, a finding consistent with the aforementioned work in which BW373U86 was the training drug (Comer et al., 1993). Morphine and U69,593 (mu and kappa agonists, respectively) produced only saline-appropriate responding. The findings that DPDPE generalized to DSLET and deltorphin II is consistent with the fact that DPDPE's stimulus control was based on its agonist activity at the delta receptor. The failure of BW373U86 to substitute for DPDPE again may be a function of the general low selectivity of BW373U86 for the mu and delta receptor subtypes (Negus et al., 1996; Picker and Cook, 1998).

One problem with the assessment of stimulus control by delta agonists has been the general unavailability of systemically active, highly selective delta compounds. Such an unavailability has resulted in the assessment primarily of intracerebroventricularly administered delta peptides (e.g., DPDPE) or nonselective, systemic alkaloids such as BW373U86. Recently, a systemically active, highly selective delta agonist, (+)-4-[(alpha*R*)-alpha-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide) (SNC80), the methyl ether of one enantiomer of BW373U86, has been synthesized (Bilsky et al., 1995; Calderon et al., 1994), which allows for a further assessment of the discriminative stimulus properties of delta activity. In this context, Brandt et al. (1999) have reported the acquisition of discriminative control in monkeys trained to discriminate intramuscular SNC80 from saline in a food-reinforced procedure. Following acquisition of the discrimination, SNC80 generalized to other systemically active piperazinyl benzamide delta agonists, e.g., SNC86, SNC162, and SNC243A, but failed to generalize to the (−)-enantiomer of SNC80, opioids selective for mu and kappa receptor subtypes or several nonopioid compounds, i.e., cocaine and ketamine. That these generalization patterns reflected the delta mediation of SNC80 control were further supported by the finding that the delta antagonist naltrindole competitively blocked SNC80's stimulus effects while the mu antagonist quadazocine was without effect.

Together, the work with DPDPE, BW373U86, and SNC80 all point to the ability of delta agonists to serve as stimuli in drug discrimination learning (DDL). To date, the work with these delta agonists has been limited to pigeons and monkeys, i.e., there have been no assessments of delta stimulus control in rodents, although rats have been used to assess the ability of delta agonists to substitute for morphine when morphine was used as the training drug (Locke and Holtzman, 1986; Spina et al., 1998; Takita et al., 1997). Given that much of the work in the literature about the discriminative stimulus properties of opioids has been done in rats (Broqua et al., 1998; Gianutsos and Lal, 1975, 1976; Grabus et al., 1999; Hill et al., 1971; Locke et al., 1989; Negus et al., 1990; Picker et al., 1990; Pournaghash and Riley, 1993; Riley and Pournaghash, 1995; Shannon and Holtzman, 1976, 1977a,b, 1979; Smurthwaite and Riley, 1994; Stevenson et al., 1992, 2000; Winter, 1975; Young et al., 1992) and that species differences in the discriminative properties (and other effects) of opioids with relative selectivity for mu and kappa receptors (Herling et al., 1980; Jarbe, 1978; Picker and Dykstra, 1987), as well as those with mixed action at these receptor subtypes (Grabus et al., 1999; Picker, 1994), have been reported, the present study assessed the establishment of discriminative control with SNC80 in rats. Specifically, rats were trained to discriminate the intraperitoneal administration of 5.6 mg/kg SNC80 from distilled water within the taste aversion baseline of DDL (Grabus et al., 1999; Mastropaolo et al., 1989; Pournaghash and Riley, 1993; Riley, 1997; Stevenson et al., 2000). Following acquisition of the discrimination and the determination of its temporal characteristics, the ability of various compounds with selectivity for mu (morphine) and delta (SNC162) receptors to substitute for SNC80 was assessed. Finally, animals were administered naltrexone and naltrindole concurrent with SNC80 to assess their ability to block the stimulus properties of SNC80.

2. General method

2.1. Subjects

The subjects were 12 experimentally naïve, female rats of Long–Evans descent, weighing approximately 200–250 g at the start of the experiment. They were housed in individual wire-mesh cages and were maintained on a 12-h light/12-h dark cycle and at an ambient temperature of 23°C. Subjects received restricted access to fluid for the

duration of the study, but they were maintained on ad-libitum access to food (Prolab Rat, Mouse, Hamster 3000).

2.2. Drugs

SNC80 and SNC162 (generously supplied by the National Institute of Diabetes and Digestive and Kidney Diseases, NIDDK) were prepared as a base dissolved in distilled water and 6 M HCl. Morphine sulfate (generously supplied by the National Institute on Drug Abuse), naloxone hydrochloride (generously supplied by DuPont Pharmaceuticals), and naltrindole (generously supplied by NIDDK) were dissolved in distilled water. All drugs were injected intraperitoneally and prepared at the following concentrations: SNC80 (2 mg/ml), morphine (4 mg/ml), SNC162 (2 mg/ml), naltrindole (2 mg/ml), naloxone (1 mg/ml).

2.3. Procedure

2.3.1. Phase I: Conditioning

Following 24 h of water deprivation, subjects were given 20-min access to water once a day for 12 consecutive days in their home cages until stable lick patterns developed. On Days 13–15 (saccharin habituation), a novel saccharin solution (0.1% w/v sodium saccharin, Sigma Pharmaceuticals) replaced water during the 20-min fluid-access period. On the last day of saccharin habituation, subjects were given an intraperitoneal injection of distilled water (2.8 ml/kg) 20 min prior to saccharin access.

On Day 16, conditioning began. All subjects were injected with 5.6 mg/kg of SNC80 20 min prior to the 20-min access to saccharin. Immediately following saccharin access, subjects were ranked according to saccharin consumption (i.e., from lowest to highest) and assigned to one of two groups (Group SL, $n=6$, and Group SW, $n=6$). Subjects in Group SL were then injected with 1.8 mEq, 0.15 M LiCl (76.8 mg/kg), while subjects in Group SW were given an equivolume injection of distilled water (i.e., the LiCl vehicle). On the following three recovery days, subjects in both groups were injected with distilled water 20 min prior to the 20-min saccharin access. No injections followed saccharin on these recovery days. This alternating procedure of a single conditioning day followed by three recovery sessions was repeated until discriminative control had been established for all experimental subjects (i.e., each subject in Group SL had consumed at least 50% less than the mean of Group SW on two consecutive conditioning trials).

2.3.2. Phase II: Generalization

The procedure during this phase was identical to that of Phase I with the following exception. On the second day following conditioning (the second recovery day within Phase I, but a probe day in this phase), subjects in Groups SL and SW were administered one of a range of doses of SNC80 (0.56–5.6 mg/kg), morphine (1.8–10 mg/kg), or SNC162 (1.8–32 mg/kg) 20 min prior to saccharin access. On any specific probe day, subjects in Group SL were given an injection only if they had consumed at least 50% less than the mean of the control subjects on the two preceding conditioning trials. Doses were administered in a mixed pattern. No injections followed saccharin access on these probe days.

2.3.3. Phase III: SNC80 time course

The procedure in this phase was identical to that of Phase I with the following exception. During each probe day (the second recovery day following conditioning), subjects in Groups SL and SW were administered the training dose of SNC80 (5.6 mg/kg) 20, 60, 120, or 240 min prior to saccharin access. Subjects in Group SL were probed only if they had consumed a least 50% less than the mean of Group SW on the two preceding conditioning trials. No injections followed saccharin access on these probe days.

2.3.4. Phase IV: Antagonism

The procedure during this phase was identical to that of Phase I with the exception that on the second recovery day following each conditioning trial (probe day) animals were given a range of doses of naltrindole (1–5.6 mg/kg) or naltrexone (0.18–1.8 mg/kg) 40 and 10 min, respectively (injection times based on Spina et al., 1998; Locke and Holtzman, 1986), prior to the training dose injection of SNC80 (i.e., 5.6 mg/kg). Twenty minutes following the injection of SNC80, all subjects were given 20-min access to saccharin. No injections followed saccharin access on these probe days.

2.4. Data analysis

Statements of statistical significance are based on a repeated-measures ANOVA for all between-group and within-group comparisons of saccharin consumption. The Newman–Keuls test post-hoc analysis was used for multiple comparisons. Student's t tests were used for between-groups comparisons in single-dose studies. The accepted level of significance for all tests was $P \leq .05$.

3. Results

3.1. Phase I: Conditioning

Fig. 1 presents the mean amount (±S.E.M.) of saccharin consumption for Groups SL and SW during water baseline (W) and saccharin habituation (S) and throughout the first seven conditioning cycles during this phase. As illustrated, there were no significant differences in fluid consumption between groups during water baseline ($t_{10}=1.059$, $P=.321$) or saccharin habituation ($t_{10}=0.496$, $P=.631$). A repeated-measures ANOVA revealed significant main effects of Group [$F(1,10)=123.894$, $P=.000$], Conditioning [$F(6,60)=39.429$, $P=.000$], and the Group

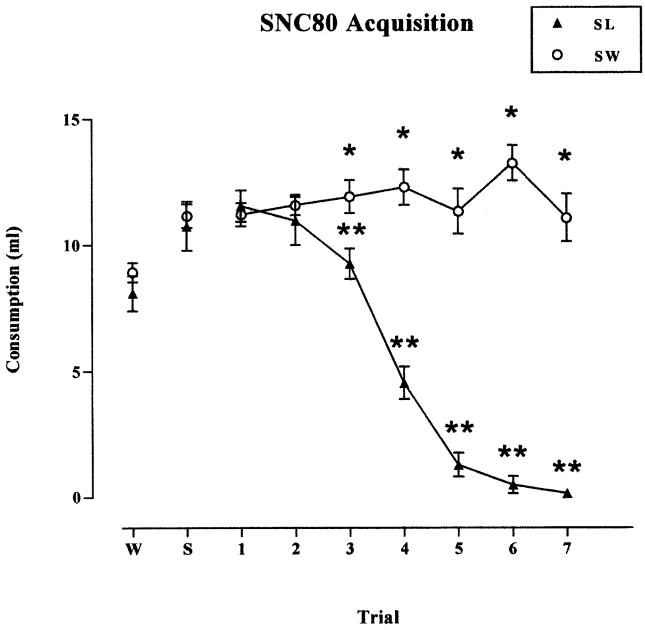

Fig. 1. Mean amount (±S.E.M.) of saccharin consumption for subjects in Groups SL and SW during water baseline (W) and saccharin habituation (S) and throughout the first seven conditioning cycles during conditioning. * Significantly different between Groups SL and SW. ** Significantly different from Trial 1 for Group SL.

× Conditioning interaction [$F(6,60) = 46.305$, $P = .000$] during acquisition of the drug discrimination. On the first conditioning trial, there were no differences in saccharin consumption between Groups SL and SW with subjects in both groups drinking approximately 11.5 ml.

For subjects in Group SL, there were no changes in consumption from the first to the second conditioning trial. By Trial 3, however, consumption significantly decreased below the level on the initial trial. Consumption remained significantly reduced throughout this phase. For subjects in Group SW, there were no changes in saccharin consumption over conditioning, with consumption approximating habituation levels on each trial. Although subjects in Groups SL and SW did not differ in saccharin consumption on Conditioning Trials 1 and 2, subjects in Group SL significantly reduced consumption of saccharin relative to that in Group SW by the third conditioning trial. This difference was maintained over conditioning. On the final conditioning trial of this phase, subjects in Groups SL and SW drank 0.16 and 11.08 ml, respectively. During recovery sessions, consumption for both groups remained high, approximating habituation levels (data not shown).

3.2. Phase II: Generalization

3.2.1. SNC80

Fig. 2 (top panel) presents the mean amount (±S.E.M.) of saccharin consumption for Groups SL and SW during conditioning (C) and recovery (R) and following various doses of SNC80. Subjects in Group SL drank significantly less

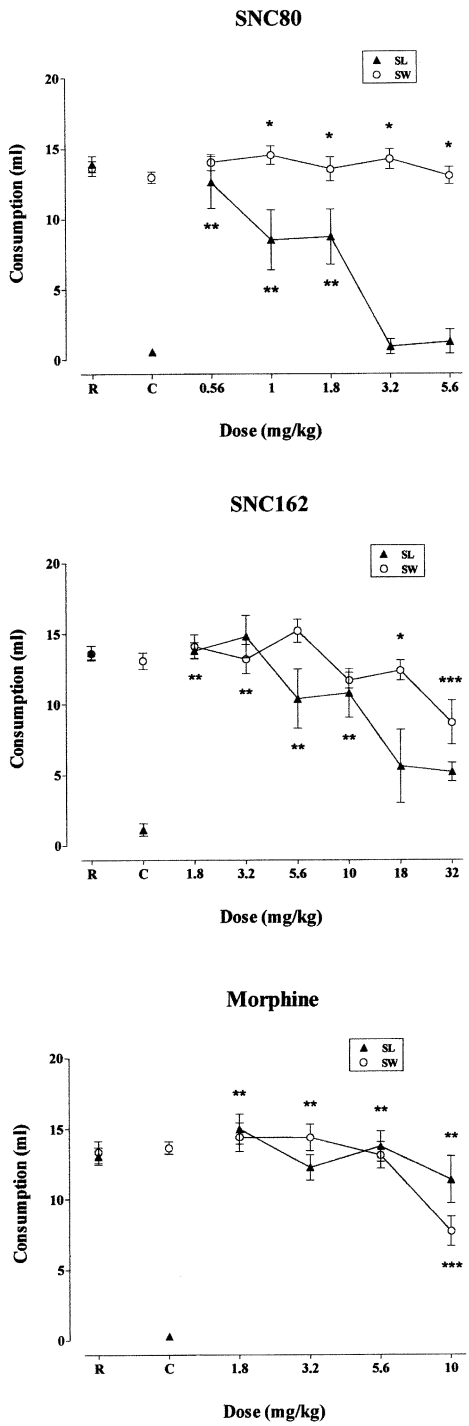

Fig. 2. Mean amounts (±S.E.M.) of saccharin consumption for subjects in Groups SL and SW during recovery (R) and conditioning (C) in this phase and following various doses of SNC80 (0.56–5.6 mg/kg, top panel), SNC162 (1.8–32 mg/kg, middle panel) and morphine (1.8–10 mg/kg, bottom panel). *Top panel:* * Significantly different between Groups SL and SW. ** Significantly different from consumption at 3.2 and 5.6 mg/kg for Group SL. *Middle panel:* * Significantly different between Groups SL and SW. ** Significantly different from consumption at 18 and 32 mg/kg for Group SL. *** Significantly different from consumption at 1.8–18 mg/kg for Group SW. *Bottom panel:* ** Significantly different from consumption at conditioning for Group SL. *** Significantly different from consumption at 1.8, 3.2, and 5.6 mg/kg for Group SW.

following conditioning than recovery, whereas subjects in Group SW displayed no significant differences in consumption between conditioning and recovery, indicating maintenance of discriminative control in this phase. A repeated-measures ANOVA revealed that there were significant main effects of Group [$F(1,10) = 48.26$, $P = .000$], Dose [$F(4,40) = 11.12$, $P = .000$], and the Group × Dose interaction [$F(6,60) = 9.149$, $P = .000$]. For subjects in Group SL, there was an inverse relationship between the dose of SNC80 and the amount of saccharin consumed (i.e., as the dose of SNC80 increased, the amount of saccharin consumed decreased). Consumption at 0.56, 1, and 1.8 mg/kg was significantly greater than consumption during conditioning. There were no significant differences in consumption between 3.2 and 5.6 mg/kg and conditioning. For subjects in Group SW, there were no consistent changes in saccharin consumption over the increasing doses of SNC80. Further, consumption at these doses approximated consumption during conditioning. At 1, 1.8, 3.2, and 5.6 mg/kg, subjects in Group SL drank significantly less than subjects in Group SW.

3.2.2. SNC162

Fig. 2 (middle panel) presents the mean amount (±S.E.M.) of saccharin consumption for Groups SL and SW during conditioning (C) and recovery (R) and following various doses of SNC162. During these probes, subjects in Group SL drank significantly less following conditioning than recovery, whereas subjects in Group SW displayed no significant differences in consumption between conditioning and recovery. There were significant main effects of Group [$F(1,8) = 5.696$, $P = .044$], Dose [$F(5,40) = 9.75$, $P = .000$] and the Group × Dose interaction [$F(5,40) = 2.911$, $P = .025$]. As illustrated, for subjects in Groups SL and SW, there was an inverse relationship between the dose of SNC162 and the amount of saccharin consumed. For subjects in Group SL, consumption at 1.8, 3.2, 5.6, and 10 mg/kg was significantly greater than consumption during conditioning. There were no significant differences in consumption between 18 and 32 mg/kg and conditioning. For subjects in Group SW, consumption at 32 mg/kg was significantly less than consumption during conditioning. There were no significant differences in consumption between conditioning and the other five lower doses. At 18 mg/kg, subjects in Group SL drank significantly less than subjects in Group SW. There were no significant differences in consumption between Groups SL and SW at the remaining doses.

3.2.3. Morphine

Fig. 2 (bottom panel) presents the mean amount (±S.E.M.) of saccharin consumption for subjects in Groups SL and SW during conditioning (C) and recovery (R) and following various doses of morphine. During these probes, subjects in Group SL drank significantly less following conditioning than recovery, whereas subjects in Group SW displayed no significant differences in consumption between conditioning and recovery. A repeated-measures ANOVA revealed a significant main effect of dose [$F(3,30) = 9.989$, $P = .000$], but not of group [$F(1,9) = 0.487$, $P = .501$] or Group × Dose interaction [$F(4,36) = 2.812$, $P = .056$]. For subjects in Groups SL, there were no consistent changes in saccharin consumption over the increasing doses of morphine. Consumption at all doses was significantly greater than consumption during conditioning. For subjects in Group SW, there was an inverse relationship between the dose of morphine and the amount of saccharin consumed. Consumption at 10 mg/kg was significantly less than consumption during conditioning. There were no significant differences between Groups SL and SW at any dose of morphine tested.

3.3. Phase III: SNC80 time course

Fig. 3 presents the mean amount (±S.E.M.) of saccharin consumption for Groups SL and SW following 5.6 mg/kg SNC80 given 20 (baseline interval), 60, 120, and 240 min prior to saccharin access. As illustrated, there were significant main effects of Group [$F(1,8) = 65.979$, $P = .000$], Time [$F(3,24) = 13.332$, $P = .000$], and the Group × Time interaction [$F(3,24) = 6.627$, $P = .002$]. For Group SL, there was a direct relationship between the preexposure interval of the training dose of SNC80 and the amount of saccharin consumed (i.e., as the preexposure interval increased, the amount of saccharin consumed increased). For subjects in Group SL, consumption at 20 and 60 min was significantly less than consumption at 240 min. For subjects in Group SW, there were no consistent changes in saccharin consumption over the increasing preexposure intervals. Subjects in Group SL drank significantly less saccharin than subjects in

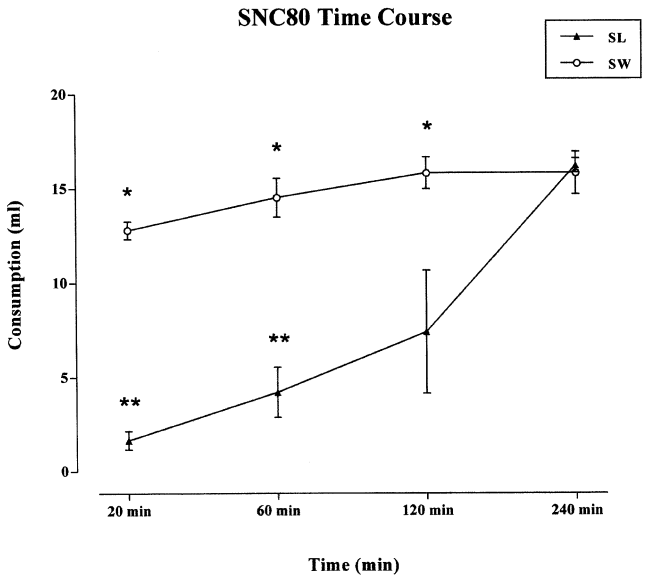

Fig. 3. Mean amount (±S.E.M.) of saccharin consumption for Groups SL and SW following 5.6 mg/kg SNC80 given 20, 60, 120, and 240 min prior to saccharin access. * Significantly different between Groups SL and SW. ** Significantly different from consumption at 120 and 240 min for Group SL.

Group SW at 20, 60, and 120 min. At 240 min, Groups SL and SW did not differ in saccharin consumption, indicating loss of discriminative control at this preexposure interval.

3.4. Phase IV: Antagonism

3.4.1. Naltrindole

Fig. 4 (top panel) presents the mean amount (±S.E.M.) of saccharin consumption for subjects in Groups SL and SW during conditioning (C) and recovery (R), as well as following the combination of various doses of NTI (1–5.6 mg/kg) administered 40 min prior to the training dose of 5.6 mg/kg SNC80 (a pilot study in our laboratory demonstrated that antagonism of SNC80 by NTI was greatest at 40-min preexposure; administration at 10 min was ineffective while pretreatment at 20 min produced partial antagonism, data not shown). During this phase, subjects in Group SL drank significantly less following conditioning than recovery, whereas subjects in Group SW displayed no significant differences in consumption between conditioning and recovery. A repeated-measures ANOVA revealed significant main effects of Group [$F(1,8) = 19.167$, $P = .002$], Dose [$F(3,24) = 20.887$, $P = .000$], and the Group × Dose interaction [$F(3,24) = 22.562$, $P = .000$] when NTI was injected prior to SNC80. For subjects in Group SL, consumption following the combination of 1 mg/kg NTI and SNC80 was not significantly different than consumption following SNC80 alone (C) but was significantly different from consumption during recovery (R). For these same subjects, consumption following the combination of 1.8, 3.2, and 5.6 mg/kg NTI and SNC80 was significantly greater than consumption following SNC80 alone and the combination of 1 mg/kg NTI and SNC80. For subjects in Group SW, there were no consistent changes in saccharin consumption over the increasing doses of NTI. Subjects in Group SL drank significantly less than subjects in Group SW at 1 mg/kg. There were no significant differences in consumption between Groups SL and SW at the three remaining doses (1.8, 3.2, and 5.6 mg/kg), indicative of complete antagonism of SNC80's discriminative effects at these three doses.

3.4.2. Naltrexone

Fig. 4 (bottom panel) presents the mean amount (±S.E.M.) of saccharin consumption for subjects in Groups SL and SW during conditioning (C) and recovery (R) in this phase, as well as following the combination of various doses of naltrexone (0.18–1.8 mg/kg) administered 10 min prior to the training dose of SNC80 (5.6 mg/kg) (see Walker et al., 1994). As above, subjects in Group SL drank significantly less following conditioning than recovery, whereas subjects in Group SW displayed no significant differences in consumption between conditioning and recovery. There were significant main effects of Group [$F(1,8) = 246.117$, $P = .000$], Dose [$F(4,32) = 4.095$, $P = .009$], and the Group × Dose interaction [$F(4,32) = 6.436$, $P = .001$] when naltrexone was injected prior to SNC80. For subjects in Group SL, consumption following SNC80 alone (C) and in combination with all doses of naltrexone did not differ. Consumption following recovery (R) was significantly greater than that following all doses of naltrexone in combination with SNC80. For subjects in Group SW, consumption following SNC80 alone was significantly greater than consumption following the combination of all

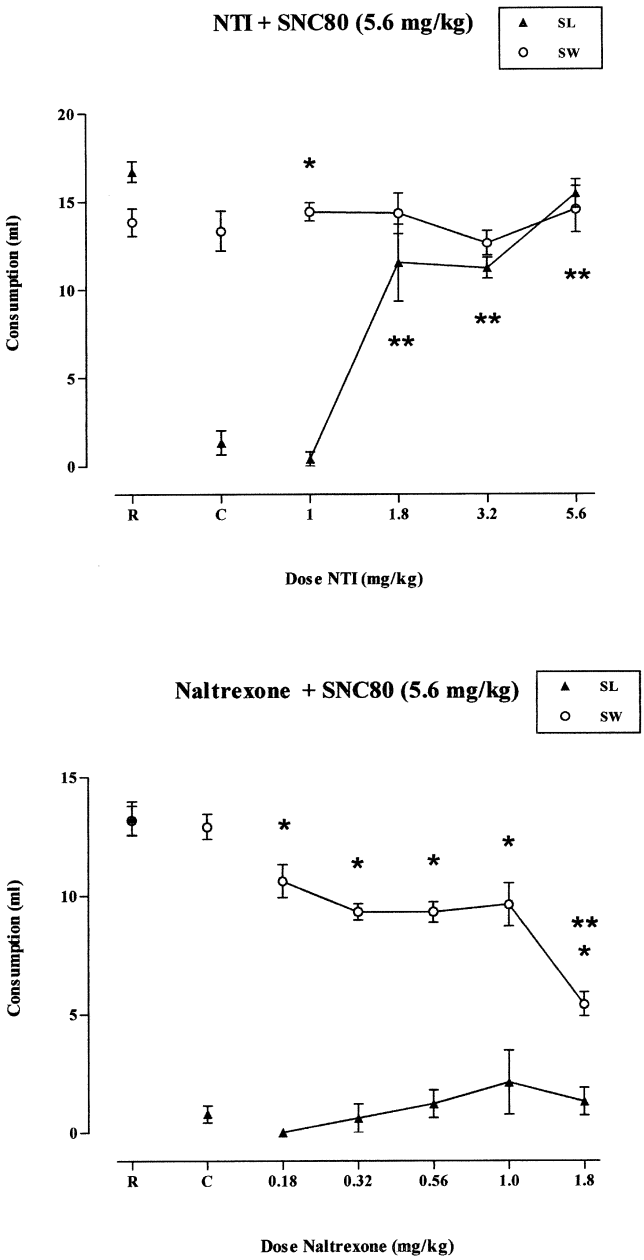

Fig. 4. Mean amount (±S.E.M.) of saccharin consumption for subjects in Groups SL and SW during recovery (R) and conditioning (C) in this phase and following NTI or naltrexone, SNC80 alone as well as the NTI/SNC80 (top panel) and naltrexone/SNC80 (bottom panel) combinations. *Top panel:* * Significantly different between Groups SL and SW. ** Significantly different from consumption at 1 mg/kg for Group SL. *Bottom panel:* * Significantly different between Groups SL and SW. ** Significantly different from consumption at 0.18–1 mg/kg for Group SW.

doses of naltrexone and SNC80. Consumption at 1.8 mg/kg naltrexone was significantly less than consumption at the other lower doses, indicating the unconditioned suppressive effects of naltrexone on fluid consumption. Subjects in Group SL drank significantly less than subjects in Group SW following all combinations of naltrexone and SNC80.

4. Discussion

The majority of reports assessing opioid DDL have concentrated on characterizing the stimulus properties of compounds selective for mu and kappa opioid receptors (see Introduction). Although generally limited to these opioid receptor subtypes, recently, compounds selective for delta opioid receptors have been assessed within the DDL design. In such assessments, stimulus control has been established. Further, the control appears selective for the delta receptor as indicated by its selective generalization to other delta agonists and selective antagonism by delta antagonists.

To date, these assessments have been limited to the monkey (Brandt et al., 1999; Negus et al., 1994, 1998) and pigeon (Comer et al., 1993; Jewett et al., 1996; Negus et al., 1996; Picker and Cook, 1998). No assessment of delta stimulus control has been examined in rodents. To that end, the present experiment examined discriminative control by the selective delta agonist SNC80 in rats and its generalization to and antagonism by compounds relatively selective for the delta and mu receptor subtypes. As described, animals acquired the discrimination between 5.6 mg/kg SNC80 and distilled water within approximately seven conditioning cycles, avoiding saccharin consumption when it was preceded by an injection of SNC80 and consuming the same saccharin solution when it was preceded by vehicle. The discriminative effects of SNC80 were maximal at 20 min, partial at 120 min, and lost at 240 min. The discrimination was dose dependent in that as the dose of SNC80 increased, the amount of saccharin consumed decreased. In subsequent generalization tests, the delta agonist SNC162 produced SNC80-appropriate responding at a dose of 18 mg/kg. Although generalization of SNC80 stimulus control was evident at 18 mg/kg of SNC162, mean consumption at this dose was higher than the mean consumption following the training dose of SNC80, i.e., 10 mg/kg, an effect consistent with at least one other report demonstrating that the effects of SNC162 are sometimes smaller and more variable than those of SNC80 (Negus et al., 1998). Assessments with higher doses of SNC162 could result in full generalization of SNC80's stimulus effects; however, as described, with higher doses there was marked unconditioned suppression of consumption in control animals (see Stevenson et al., 2000, for similar dose-response suppression with methadone). Thus, it is unknown if complete generalization could be produced with SNC162 (although see Brandt et al., 1999, for complete generalization in monkeys). While SNC162 partially substituted for SNC80, the mu agonist morphine produced vehicle-appropriate responding at all doses tested. Although these selective generalization patterns with SNC162 and morphine suggest that the discriminative effects of SNC80 were mediated at the delta, but not the mu, receptor, assessments with other delta and mu agonists within this preparation are needed before general conclusions can be made about the mediation of SNC80's stimulus effects. As noted, SNC80's discriminative control was completely blocked by the delta-selective antagonist NTI, but not by the mu-selective antagonist naltrexone, again suggestive of delta mediation of SNC80's stimulus effects. However, it is possible that had higher doses of naltrexone been given, antagonism of SNC80's discriminative effects might have been produced. Although possible, the dose range examined is highly effective in antagonizing mu stimulus control (Locke and Holtzman, 1986). Further, with the highest dose assessed (i.e., 1.8 mg/kg), generalized behavioral suppression was evident in the control subjects, precluding interpretation of any possible antagonism of SNC80 discriminative control. The dose range examined for NTI was also within the range of doses effective in antagonizing delta-mediated effects (see Spina et al., 1998). As described, in the present experiment antagonism was evident at a dose as low as 1.8 mg/kg.

As previously described, rats readily discriminate both mu- and kappa-selective agonists from their respective vehicles. The present findings indicate that rats also discriminate compounds that are selective for the delta receptor subtype, thus extending the class of compounds that can serve such discriminative functions for the rat (see also Platt et al., 1999; Stevenson et al., 2000; see Locke and Holtzman, 1986; Picker, 1997; Ukai and Holtzman, 1988 for instances of delta substitution for mu stimulus control). The fact that rats can use delta receptor activity as a discriminative stimulus and that these stimulus effects are different from those generated by activity at the mu receptor might be expected given that compounds selective for mu and delta receptors have been shown to produce different behavioral effects within a variety of behavioral and physiological preparations (though see Picker and Cook, 1998). For example, although mu and delta agonists are both effective analgesics, they differ markedly in their antinociceptive profiles. Specifically, the mu agonist morphine is effective in reversing both chronic thermal hyperalgesia and acute nociception, whereas the delta agonist SNC80 inhibits chronic hyperalgesia only (Fraser et al., 2000; see also Brandt et al., 1999, for similar differences in the monkey). Moreover, whereas mu agonists produce respiratory depression, delta agonists generally do not (Su et al., 1998; Takita et al., 1997). More specifically, DAMGO has been shown to reduce respiratory frequency, inspiratory duration and C4 (ventral root) amplitude, whereas DPDPE has no significant effects on these measures (although DPDPE does decrease respiratory frequency in some preparations; see Takita et al., 1997). Further, in a recent paper assessing systemically active delta compounds in the conditioned taste aversion

design, Hutchinson et al. (2000) demonstrated that SNC80 was effective in inducing a moderate to strong taste aversion, whereas morphine produced a weak aversion and also induced these aversions at a slower rate than SNC80.

The present findings indicating that rats (like pigeons and monkeys) discriminate compounds relatively selective for the delta receptor subtype also extend the variety of species for which such control can be established. Interestingly, the control established with SNC80 (as well as its generalization and antagonism) in the present paper is quite similar to that seen with pigeons and monkeys trained to discriminate delta-selective agonists from vehicle. For example, in relation to the generalization of delta agonist stimulus control, Jewett et al. (1996) demonstrated that pigeons trained to discriminate 100 μg of DPDPE from saline generalized this control to a variety of delta agonists, e.g., DSLET and deltorphin II, but not to the mu agonists morphine and DAMGO. More relevant to the present findings, Brandt et al. (1999) demonstrated that monkeys trained to discriminate 0.32 mg/kg of SNC80 from saline generalized SNC80 control to the delta agonists SNC80, SNC162, SNC86, and SNC243A, but not to the mu agonists morphine and fentanyl. Interestingly, the time course of SNC80 control in the rat paralleled that previously reported by Brandt et al. with the monkey. Specifically, for both rat and monkey, SNC80 control was maximal at 15 min, still evident at 60 min, and absent after 240 min. Given that the time course of delta control has not been assessed with the pigeon (not with SNC80 nor any delta agonist), it is not known to what extent these patterns with the rat and monkey generalize to the pigeon. The similarity among the rat, pigeon, and monkey was also evident with the selective antagonism of delta control. As described, in the present paper the delta antagonist NTI, but not the mu antagonist naltrexone, completely blocked SNC80's stimulus effects in rats. In earlier reports, DPDPE's stimulus effects in pigeons were selectively blocked by NTI, while SNC80's stimulus effects in monkeys were completely blocked by NTI, but not by the mu antagonist β-FNA (Brandt et al., 1999; for similar antagonism of the non-selective delta agonist, BW373U86, see Comer et al., 1993). Although the stimulus properties of delta agonists appear similar across species, these comparisons are based on only a few reports and until more complete parametric assessments of delta control are made, conclusions regarding delta control should be cautiously made.

The abovementioned similarities in the stimulus properties of delta agonists across species seem somewhat inconsistent with the reported differences in the discriminative properties of mu and kappa agonists among the monkey, rat, and pigeon (Herling et al., 1980; Picker and Dykstra, 1987, 1989; Schaefer and Holtzman, 1978). For example, most reports demonstrate that both monkeys and rats can discriminate between the stimulus properties of mu and kappa agonists of varying efficacy, whereas the pigeon typically can only discriminate between mu and more highly selective kappa agonists (Picker and Dykstra, 1987). Although suggestive of species differences, these reported differences may be less a function of species than of the specific parameters under which the discrimination was made. For example, Shannon and Holtzman (1979) demonstrated that rats trained on a high dose of morphine (5.6 mg/kg) did not generalize control to amphetamine or cyclazocine and only partially generalized it to nalbuphine, whereas, rats trained on a low dose of morphine (1.75 mg/kg) generalized morphine control to the mixed opioid nalbuphine, the nonopioid amphetamine and, partially, to the kappa agonist cyclazocine. Similarly, Picker et al. (1990) reported that rats trained on a high dose of morphine (10 mg/kg) generalized stimulus control to a variety of mu agonists (i.e., methadone, morphine, and fentanyl) but not to the kappa agonists ketocyclazocine, bremazocine, and ethylketocyclazocine nor to the nonopioid amphetamine. Conversely, rats trained on a low dose of morphine (3.0 mg/kg) completely generalized stimulus control to the mu agonists and the kappa agonist ketocyclazocine and partially to the kappa agonists bremazocine and ethylketocyclazocine. In a report with pigeons, Picker (1994) demonstrated that the mixed-action opioids nalbuphine, levallorphan, nalorphine, (−)-cyclazocine, (−)-NANM, and cyclorphan did not substitute for a high (0.18 mg/kg) dose of fentanyl but completely substituted for a low (0.056 mg/kg) dose of fentanyl (see Grabus et al., 1999, for similar analyses with mixed action opioids). Although the above reports do not demonstrate that species differences do not exist, they do suggest that the training dose is a critical factor in characterizing mu and kappa discriminative control. However, further research on the possible training dose effects of delta agonists is needed before one can conclude that training dose is an important factor in characterizing delta opioid stimulus control.

In summary, the present study demonstrated delta control for the first time in rodents with the systemically active delta agonist SNC80. The selective generalization and antagonism patterns in the present study suggest that this control is mediated at the delta, but not the mu, receptor. However, given that this report is one of only a few that assess the discriminative effects of systemically active delta agonists, further parametric assessments are necessary to completely characterize delta stimulus control.

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