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Cannabinoid modulation of rat pup ultrasonic vocalizations

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Abstract

The present study investigated the effects of the cannabinoid receptor agonist CP 55,940 ((−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol) and the cannabinoid receptor antagonist SR 141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride) on ultrasonic vocalizations, body temperature and activity in 11–13-day-old rat pups. Testing occurred in a 5-min session 30 min following drug administration. CP 55,940 produced a dose-dependent decrease in ultrasonic vocalizations, with a 1000-μg/kg dose causing an almost complete inhibition of calls. Doses of 100 and 1000 μg/kg of CP 55,940, but not 10 μg/kg, caused significant hypothermia in the pups and the 1000-μg/kg dose also inhibited activity. The cannabinoid receptor antagonist SR 141716A (20 mg/kg) reversed the effects of 1000 μg/kg CP 55,940 on ultrasonic vocalizations and body temperature, but the benzodiazepine receptor antagonist flumazenil (20 mg/kg), the dopamine D_1 receptor antagonist SCH 23390 (0.5 mg/kg) and the opioid receptor antagonist naloxone (1 mg/kg) did not. When administered alone, SR 141716A (20 mg/kg) increased pup ultrasonic vocalizations without affecting body temperature or activity. These results indicate that cannabinoids modulate ultrasonic vocalization production in rat pups in a manner that is independent of hypothermia. The increase in ultrasonic vocalizations produced by SR 141716A is one of the first reported behavioural effects of this drug and suggests that the endogenous cannabinoid ligand anandamide may be involved in the regulation of ultrasonic vocalizations.

Keywords: Cannabinoid; Cannabis; Ultrasonic vocalization; Anxiety; SR 141716A; CP 55,940

1. Introduction

Infant rodents, when isolated from their mother and littermates emit characteristic vocalizations in the ultrasonic range (Hofer and Shair, 1978; Oswalt and Meier, 1975; Zippelius and Schleidt, 1956). These ultrasonic vocalizations serve a communicatory function, acting as a stimulus for maternal search and retrieval, as well as for nest building and maternal grooming (Brouette Lahlou et al., 1992; Brunelli et al., 1994; Smotherman et al., 1978). The reduction in body temperature caused by removal of the pup from the nest is an important stimulus for ultrasonic vocalization emission, since as ambient temperature and the body temperature of the pup decrease, the number of ultrasonic vocalizations increase (Blumberg et al., 1992; Oswalt and Meier, 1975).

The emotional state of the pup is also a determinant of ultrasonic vocalization production. For example, ultrasonic vocalizations in 14-day-old pups are suppressed by the presence of an unfamiliar (and possibly infanticidal) adult male rat, a response that is seen in combination with behavioural inhibition in the pup (Takahashi, 1992; Takahashi, 1994). Further, anxiogenic drugs, such as pentylenetetrazole, increase ultrasonic vocalization emission in rat pups without producing appreciable hypothermia (Carden et al., 1993). In contrast, anxiolytic drugs, such as benzodiazepines, buspirone and the neuroactive steroid allopregnanolone, potently reduce isolation-induced ultrasonic vocalizations (Carden and Hofer, 1990; Miczek et al., 1995; Winslow and Insel, 1991b; Zimmerberg et al., 1994). These data have lead to the proposal that the measurement of ultrasonic vocalizations in isolated pups may provide a measure of anxiety (Miczek et al., 1995; Winslow and Insel, 1991a).

However, not all drugs that reduce ultrasonic vocalizations in pups are necessarily anxiolytic. Drugs with positively reinforcing properties, such as cocaine (Barr and Wang, 1993), 3,4-methylenedioxymethamphetamine

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(MDMA) (Winslow and Insel, 1990) and morphine (Carden and Hofer, 1990), all reduce pup ultrasonic vocalizations. In addition, we have recently shown that D_1, D_2 and D_3 dopamine receptor agonists, all of which have positively reinforcing effects in adult rats, produce a powerful inhibition of pup ultrasonic vocalizations (Brown et al., 1995). Conversely, drugs that have aversive effects in adult rats (as shown in the conditioned place preference paradigm), such as the κ-opioid receptor agonist U50,488, increase pup ultrasonic vocalizations (Carden et al., 1994). From this, it might be suggested that a reduction in isolation-induced ultrasonic vocalizations may indicate either a positively reinforcing (euphorogenic) or negatively reinforcing (anxiolytic) effect of a drug.

One of the most prevalent mood-altering drugs used by humans is cannabis. Our understanding of the neural effects of cannabis has been greatly increased by the recent discovery and cloning of the brain cannabinoid receptor (Matsuda et al., 1990) and the isolation of an endogenous ligand for this receptor called anandamide (Devane et al., 1992). Cannabinoid receptors have a widespread distribution in the rat brain and are present at relatively high densities in the forebrain of the neonatal rat (McLaughlin and Abood, 1993). Given the well-documented mood-altering effects of cannabis, it was predicted here that cannabinoids will modulate emission of pup ultrasonic vocalizations. However, the direction in which this modulation may occur is hard to predict. Unlike other drugs that humans self-administer, such as opiates and cocaine, cannabinoids are often found to have aversive effects in rats, producing conditioned place aversions (McGregor et al., 1996; Parker and Gillies, 1995), an anxiogenic effect on the elevated plus maze (Onaivi et al., 1990) and a failure to support self-administration (Corocoran and Amit, 1974; Leite and Carlini, 1974). This would suggest that cannabinoids, like other aversive or anxiogenic drugs, may increase pup ultrasonic vocalizations. However, a positively reinforcing effect of cannabinoids in rats is sometimes reported in the literature, for example with Lewis rats in the self-stimulation paradigm (Gardner and Lowinson, 1991) and with Long-Evans rats in the conditioned place preference paradigm, albeit across a narrow dose range (Lepore et al., 1995). Thus, the exact nature of cannabinoid effects on pup ultrasonic vocalizations is hard to predict and may depend upon the dose of cannabinoid and the age and strain of rats tested.

An important new development in cannabinoid pharmacology has been the discovery of a highly specific antagonist at the cannabinoid receptor (Rinaldi-Carmona et al., 1994, 1995). This drug, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR 141716A), blocks the behavioural and physiological effects of cannabinoid receptor agonists while apparently exhibiting minimal intrinsic activity (Rinaldi-Carmona et al., 1995). The present study examined whether SR 141716A would reverse the effects of the potent selective cannabinoid receptor agonist CP 55,940 ((−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol) on pup ultrasonic vocalizations.

In addition, a range of non-cannabinoid receptor antagonists were assessed. The positively reinforcing effects of cannabinoids in the Lewis strain of rat have been found to involve an interaction with dopamine and opioid neurotransmitter systems (Gardner and Lowinson, 1991). Recent evidence also points to an interaction between cannabinoid and D_1 dopamine receptors in the cataleptic response elicited by cannabinoids in adult rats (Rodriguez de Fonseca et al., 1994). Other evidence points to an interaction between cannabinoids and brain benzodiazepine receptors, with the anxiogenic effect of cannabinoids in mice prevented by administration of the benzodiazepine antagonist flumazenil (Onaivi et al., 1990). Flumazenil has also been reported to reverse the coma induced in human infants through accidental ingestion of large quantities of cannabis (Rubio et al., 1993). In the present experiment, therefore, we examined the ability of the benzodiazepine receptor antagonist flumazenil, the opioid receptor antagonist naloxone and the dopamine D_1 receptor antagonist SCH 23390 to block the effects of the cannabinoid receptor agonist CP 55,940 on pup ultrasonic vocalizations.

2. Materials and methods

2.1. Subjects

Subjects were the offspring of mated pairs of Long-Evans Hooded rats (*Rattus norvegicus*) purchased from Charles River Canada (St. Constant, Québec, Canada) and bred at Dalhousie University (Halifax, Nova Scotia, Canada). The adults were housed in a colony room maintained at 22 ± 1°C on a 12:12 reversed light:dark cycle with lights off at 09:30 h. Pairs of rats were mated for 1 week and then the male was removed.

Following birth, each litter was housed with its mother in a Plexiglas cage (23 × 45 × 15 cm) with wood chips for bedding and shredded paper for nesting material. Purina rodent laboratory chow and water were available ad libitum. Litters were culled to 12 pups on post-natal day 1 (day 0 = day of parturition), and pups remained with their dams throughout the experiment. Pups were sexed by examination of anogenital distance and by noting the presence/absence of incipient teats.

All pups were tested once when 12 ± 1 days of age.

2.2. Apparatus

Testing for ultrasonic vocalizations and activity took place in a Plexiglas test chamber (26 × 15 × 12 cm). Ultrasonic vocalizations were recorded via an ultrasonic microphone connected to a SM2 bat detector (Ultrasound

Advice, UK). The bat detector fed its broadband output to a custom-built four-channel digitizer. This digitizer contained four variable band pass filters which could be set to particular frequencies (in this case, 28, 36, 44 and 52 kHz). When an input was detected at one of these frequencies, the digitizer produced a pulse for the duration of the signal. The output of the digitizer was connected via a terminal panel and interface card (Strawberry Tree, Sunnyvale, CA, USA) to a Macintosh 2cx computer on which a custom program written under the Strawberry Tree Workbench Mac software (McGregor, 1996) recorded the occurrence of each ultrasonic vocalization on a minute-by-minute basis.

Activity was recorded using a passive infrared motion detector ('Safe House', Radio Shack) held in place by a metal retort stand just above the test cage. The capacitor circuitry inside the motion detector was modified so that each movement of the pup triggered the detector for a constant period of 0.5 s. The output of the detector was sent via a Strawberry Tree terminal panel and interface card to the Macintosh 2cx computer where number of seconds that the pup spent moving during the 5-min test session were recorded.

2.3. Drugs

CP 55,940 (Pfizer) was initially dissolved in 100% ethanol and then diluted down to make a final solution of 2.5% ethanol, 2.5% Tween 80 and 95% saline. This vehicle solution has been found to have no detectable behavioural effects in adult rats (McGregor et al., 1996). SR 141716A (Sanofi Recherche) was dissolved in a vehicle of 5% ethanol, 5% Tween 80 and 90% saline. Flumazenil (Roche) was suspended in a solution of 2% Tween 80 in saline. Both SCH 23390 and naloxone hydrochloride (Research Biochemicals) were dissolved in saline. All drugs were injected i.p. in a volume of 10 ml/kg.

2.4. Effects of CP 55,940 administered alone

In Experiment 1, a split-litter design was used with three pups from each litter of 12 randomly allocated to each of the four treatments (giving a total of $n = 12$ per treatment). The four treatments were as follows: vehicle or CP 55,940 (10, 100 or 1000 μg/kg). Data from one pup in the 1000-μg/kg condition were discarded due to experimenter error.

The 10- and 100-μg/kg doses of CP 55,940 were chosen to match the effective behavioural doses seen in adult rats in a previous study (McGregor et al., 1996). Pilot studies indicated that these doses were submaximal in rat pups, so a higher (1000 μg/kg) CP 55,940 dose was introduced into the present study.

On the test day, the litter was brought to a pre-test room which was illuminated by a 40-Watt red light. A pup was removed from its home cage and its axillary temperature (pre-injection temperature) was measured by placing a surface temperature probe (model SST-1 copper-constantan thermocouple; Physitemp Instruments, Clifton, NJ, USA) under its right forelimb until the thermometer (Digi-Sense model 8528-20; Cole-Parmer Instruments, Chicago, IL, USA) reading stabilized. Axillary temperature was used as an indication of internal temperature because more direct methods, such as rectal probes, are more stressful, and may stimulate ultrasonic vocalizations. Furthermore, axillary temperature measurements are virtually indistinguishable from rectal temperature measurements in rodent pups (Gebczynski, 1975).

The pup was then weighed to the nearest 0.1 g and injected with the appropriate drug. After injection, the pup was marked with non-toxic ink and placed in a holding chamber that was partially submerged in a thermostatically controlled water bath maintained at 34 ± 1.0°C. At any one time, there were usually 1–3 rat pups in this holding chamber awaiting testing.

After 30 min in this chamber, the pup was removed and its axillary temperature (pre-test temperature) was measured again. The pup was then transported to the test chamber (ambient temperature of 22 ± 2°C) in a plastic cup filled with home cage wood shavings and was placed in the centre of the test chamber for a 5-min test. Pups were handled minimally, and surgical gloves were worn to minimize exposure of the pups to novel odours and to heat transfer from the experimenter.

After the test, the pup was removed from the test chamber and its axillary temperature (post-test temperature) was taken using the same procedure as before. The test chamber was cleaned with 70% ethanol and dried with paper towelling to remove any odours left by the pup.

2.5. Effects of CP 55,940 administered in combination with various antagonists

In Experiment 2, a split-litter design was used with two pups from each litter of 12 randomly allocated to each of the six treatments (giving $n = 12$ per treatment). The six treatments were as follows: vehicle + vehicle, CP 55,940 (1000 μg/kg) + vehicle, CP 55,940 (1000 μg/kg) + SR 141716A (20 mg/kg), CP 55,940 (1000 μg/kg) + flumazenil (20 mg/kg), CP 55,940 (1000 μg/kg) + SCH 23390 (0.5 mg/kg), and CP 55,940 (1000 μg/kg) + naloxone (1.0 mg/kg). Single doses of naloxone, flumazenil and SCH 23390 were chosen corresponding to the effective doses seen in previous studies of pup ultrasonic vocalizations (Brown et al., 1995; Carden and Hofer, 1990). A high dose of SR 141716A was chosen (20 mg/kg) in an attempt to ensure reversal of the effects of the relatively high dose of CP 55,940 (1000 μg/kg) being used. The two injections were given consecutively, the antagonist being given first, 30 min prior to testing. All other procedures were as reported above.

Because it was noted in the first experiment that many

pups given high doses of cp 55,940 produced squeak-like audible vocalizations when being handled on the way to or from the test chamber, the presence or absence of such vocalizations were recorded for each rat in this experiment.

2.6. Effects of SR 141716A administered alone

In Experiment 3, a split-litter design was used with six pups from each of two litters randomly allocated to one of the two treatments (giving $n = 12$ per treatment). The two treatments were as follows: vehicle or SR 141716A (20 mg/kg). All other procedures were as reported above.

2.7. Data analysis

For each pup, data on three dependent variables were analyzed. These were: (1) number of ultrasonic vocalizations emitted during the 5-min test period; (2) number of seconds the pup engaged in activity during the 5-min test session; and (3) difference in pre-test and post-test temperature (i.e. temperature after the 5-min test session minus temperature before the 5-min test).

Data for each dependent variable were analyzed using between-subjects analysis of variance with α set at 0.05. Where required, post-hoc comparisons were made using Dunnett's test (two-tailed) which compares all treatments with a control. In the first experiment, the control was the vehicle group. In the second experiment where the effects of antagonists were of interest, the designated control was the group given vehicle + CP 55,940. All analyses were performed on the SuperAnova statistical package (Abacus Concepts, Berkeley, CA, USA). Because analyses of experimental data according to sex did not yield any reliable sex differences, no discussion of gender is included in the paper.

3. Results

3.1. Effects of CP 55,940 administered alone

The data for ultrasonic vocalizations, locomotor activity and change in body temperature across the 5-min test are shown in Fig. 1. CP 55,940 produced a significant reduction in ultrasonic vocalizations [$F(3,43) = 6.833$, $P < 0.01$]. Post-hoc tests showed that both the 100- and 1000-μg/kg groups emitted significantly fewer ultrasonic vocalizations than the vehicle only group.

There was also a significant effect of CP 55,940 on activity [$F(3,43) = 3.376$, $P < 0.05$]. Post-hoc tests showed that the 1000-μg/kg group were significantly less active during the test session than the vehicle only group.

There was also a significant overall effect of CP 55,940 on the change in body temperature across the test session [$F(3,43) = 23.49$, $P < 0.01$]. Post-hoc tests showed that both the 100- and 1000-μg/kg groups showed a greater fall in body temperature over the test session than the vehicle only group.

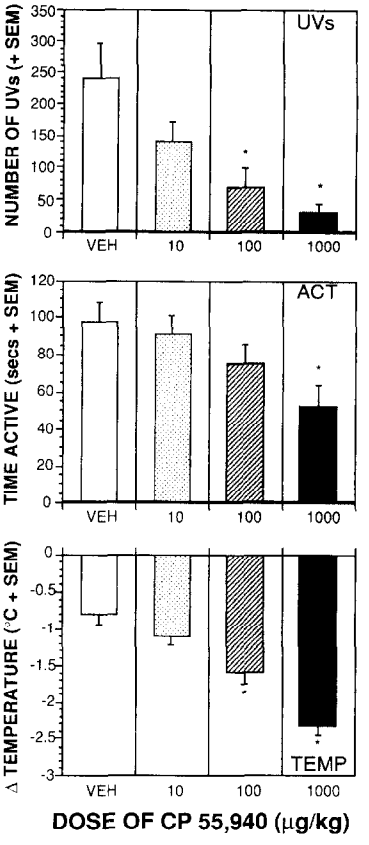

Fig. 1. Effects of 10, 100 and 1000 μg/kg CP 55,940 compared to the vehicle alone condition (VEH) on the number of ultrasonic vocalizations (UVs) and the time spent active (ACT) during the 5-min test and on the post- minus pre-test change in body temperature (TEMP). * Significantly different from vehicle group.

3.2. Effects of CP 55,940 administered in combination with various antagonists

The data for ultrasonic vocalizations, locomotor activity and change in body temperature across the 5-min test are shown in Fig. 2. With respect to ultrasonic vocalizations, there was a significant overall effect of drug treatment [$F(5,65) = 9.46$, $P < 0.01$]. Post-hoc tests showed that both the vehicle + vehicle and CP 55,940 + SR 141716A groups differed significantly from the CP 55,940 + vehicle group. Thus, SR 141716A reversed the effect of CP 55,940 on ultrasonic vocalizations, but SCH 23390, flumazenil and naloxone did not.

There was also a significant group effect with respect to activity [$F(5,65) = 12.677$, $P < 0.01$]. Post-hoc tests showed that only the vehicle + vehicle group differed significantly from the CP 55,940 + vehicle group. While there was a clear tendency for SR 141716A to reverse the suppression of activity produced by CP 55,940, this effect fell just short of significance using Dunnett's test.

There was also a significant overall effect of drug

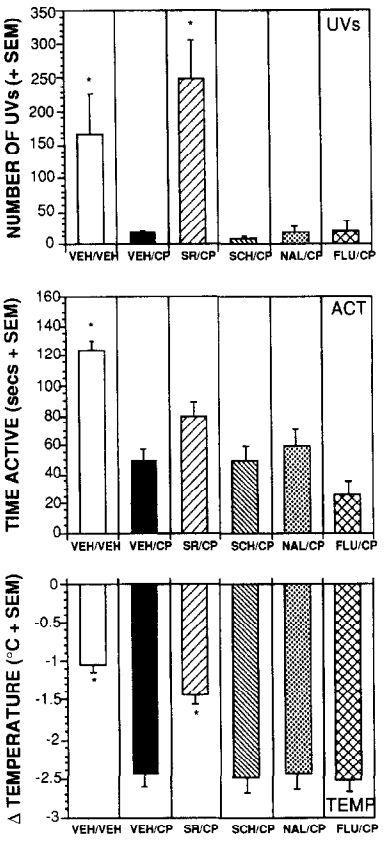

Fig. 2. The effects of CP 55,940 (1000 μg/kg) alone and in combination with SR 141716A (20 mg/kg), SCH 23390 (0.5 mg/kg), naloxone (1.0 mg/kg) or flumazenil (20 mg/kg) on the number of ultrasonic vocalizations (UVs) and the time spent active (ACT) in the 5-min test and on the post- minus pre-test change in body temperature (TEMP). VEH, vehicle; CP, CP 55,940; SR, SR 141716A; SCH,SCH 23390; NX, naloxone; FLU, Flumazenil. * Significantly different from CP 55,940 alone group.

condition on the change in body temperature across the test session [$F(5,65) = 15.84$, $P < 0.01$]. Post-hoc tests showed that SR 141716A, but not the other antagonists, reversed the drop in body temperature produced by CP 55,940.

The number of rats producing audible vocalizations during handling differed among the six groups as shown in Table 1 ($\chi^2(5) = 22.331$, $P < 0.01$). As can be seen in this table, nearly all of the rats given CP 55,940 alone produced such vocalizations while none of the vehicle

Table 1
Number of rats emitting audible vocalizations following CP 55,940 alone or in combination with various antagonists

Treatment	Rats emitting audible vocalizations/tested (n/n)
Vehicle + vehicle	0/12
CP 55,940 + vehicle	11/12
CP 55,940 + SR 141716A	4/12
CP 55,940 + SCH 23390	10/12
CP 55,940 + naloxone	8/12
CP 55,940 + flumazenil	7/12

Table 2
Effects of SR 141716A (20 mg/kg) or vehicle on ultrasonic vocalizations, activity and body temperature in rat pups

	Vehicle (mean ± S.E.M.)	SR 141716A (mean ± S.E.M.)
Ultrasonic vocalizations	242.83 ± 47.20	414.33 ± 44.07 [a]
Time active (s)	105.35 ± 11.50	87.48 ± 11.43
Temperature change (°C)	−1.58 ± 0.11	−1.25 ± 0.24

[a] $P < 0.01$.

treated rats did so ($\chi^2(1) = 16.783$, $P < 0.01$). The cannabinoid receptor antagonist SR 141716A reduced these audible vocalizations to a level no different than the vehicle control ($\chi^2(1) = 2.70$, $P = 0.10$) while the groups receiving the other antagonists did not differ from the CP 55, 940 + vehicle group ($\chi^2(3) = 2.222$, $P = 0.53$).

3.3. Effects of SR 141716A administered alone

The data for ultrasonic vocalizations are shown in Table 2. SR 141716A produced a significant increase in ultrasonic vocalizations [$F(1,22) = 7.05$, $P < 0.05$] relative to vehicle. However, it did not affect activity [$F(1,22) = 1.22$, $P > 0.2$] or body temperature [$F(1,22) = 1.64$, $P > 0.2$].

4. Discussion

The present study shows that the synthetic cannabinoid CP 55,940 caused a dose-dependent reduction of ultrasonic vocalizations in rat pups isolated from their mother and siblings in a novel environment. Notably, this reduction in ultrasonic vocalizations occurred in the presence of a substantial drug-induced hypothermia, supporting other findings that hypothermia is not a sufficient condition for the elicitation of separation-induced ultrasonic vocalizations (Hofer and Shair, 1991). These data also concur with our recent findings that the D2 agonist quinpirole and the putative dopamine D3 receptor agonist 7-OH-DPAT both reduce ultrasonic vocalizations in the presence of a robust hypothermic effect (Brown et al., 1995).

The present data also bear upon the hypothesis that anxiety is a key elicitor of ultrasonic vocalizations in pups. If this were the case, then one would have to surmise that CP 55,940 in the present study caused an anxiolytic effect. However, previous studies have shown that a dose of 100 μg/kg CP 55,940 produces a strong conditioned place aversion in adult Wistar rats (McGregor et al., 1996) and that similar doses of CP 55,940 elicit an anxiogenic effect on the elevated plus maze test (Onaivi et al., 1990). Nonetheless, a recent study has shown that certain low-to-moderate doses of Δ^9-tetrahydrocannabinol can produce a conditioned place preference in adult Long-Evans rats, suggesting that cannabinoids can have a positively reinforcing effect in this particular strain (Lepore et al., 1995).

Such an effect could potentially explain the effects of CP 55,940 in the present study, with the reduction in ultrasonic vocalizations seen as similar to that produced by other positively reinforcing drugs, such as cocaine (Barr and Wang, 1993), MDMA (Winslow and Insel, 1990) and morphine (Carden and Hofer, 1990).

One problem with this hypothesis was the finding in the present study that most pups were hyperreactive following the highest CP 55,940 dose used (1000 μg/kg), emitting audible vocalizations when picked up by the experimenter for transport to the test chamber. This hyperreactivity suggests that the CP 55,940 may have been having an aversive or anxiogenic effect in the pups that was, somewhat surprisingly, not translated into an increase in ultrasonic vocalizations. Interestingly, audible vocalizations are also consistently seen in adult rats subjected to high doses (1000 μg/kg) of CP 55,940 that appear to be aversive (I.S. McGregor, unpublished observations). This finding prevents us from embracing the hypothesis that CP 55,940 was causing a positively reinforcing effect in the present study.

One other possible explanation is that the reduction in ultrasonic vocalizations may be simply a reflection of CP 55,940-induced catalepsy or sedation. However, several factors refute this possible explanation. First, catalepsy is only seen in adult rats with high doses of CP 55,940 (> 100 μg/kg) while in the present study a clear tendency towards suppression of ultrasonic vocalizations was seen with a much smaller (10 μg/kg) dose which had negligible effects on activity (Fig. 1). Second, activity was not significantly depressed by 100-μg/kg CP 55,940 while ultrasonic vocalizations showed a highly significant decline. Third, while the cannabinoid receptor antagonist SR 141716A evidenced a complete reversal of the CP 55,940-mediated suppression of ultrasonic vocalizations, it produced only a partial (and non-significant) reversal of the activity reducing effect of CP 55,940. Finally, the fact that pups given the high dose of CP 55,940 showed audible vocalizations to touch shows that they were capable of vocalizing in the drug state.

It is also worth noting that the reduction in activity seen in pups in the present study with 100 μg/kg CP 55,940 was considerably less than that seen in adult rats at this same dose (McGregor et al., 1996). This suggests that 12-day-old rat pups may be hyporesponsive to the motor suppressive effects of cannabinoids. Responsivity might well increase in line with the 5-fold increase in the number of cannabinoid receptors seen across the first few weeks of post-natal development (Belue et al., 1995; Rodriguez de Fonseca et al., 1993).

The reversal by SR 141716A of the CP 55,940-mediated reduction of ultrasonic vocalizations and body temperature confirms other reports that this drug functions as an effective antagonist at the cannabinoid receptor (Collins et al., 1995; Rinaldi-Carmona et al., 1994, 1995; Wiley et al., 1995). Not only did SR 141716A reverse the effects of CP 55,940 on ultrasonic vocalizations but it seemed to produce an 'overshoot' effect whereby the number of ultrasonic vocalizations in pups given the agonist and antagonist together was increased relative to those given the agonist alone (see Fig. 2). This suggested a possible intrinsic effect of SR 141716A which was subsequently confirmed in the final experiment.

This behavioural effect of SR 141716A in pups is one of the first intrinsic behavioural effects of this drug to be reported. While there are two recent reports of an abstinence syndrome elicited by SR 141716A, this only occurred in rats that had been pre-exposed to daily injections of high doses of cannabinoids (Aceto et al., 1995; Tsou et al., 1995). One other study has shown that SR 141716A increases the cell population response in midbrain A9 dopaminergic neurons without affecting their spontaneous firing rate (Gueudet et al., 1995). However, the possible behavioural correlates of such an effect are unclear.

The mechanism underlying the SR 141716A stimulation of pup ultrasonic vocalizations can only be speculated upon. One possibility is that the recently discovered endogenous cannabinoid, known as anandamide (Devane et al., 1992), acts as an inhibitor of ultrasonic vocalizations in pups under conditions of isolation, and that SR 141716A blocks this inhibitory effect of anandamide to produce a disinhibition of ultrasonic vocalizations. Clearly, there must be some neurochemical mechanism underlying the inhibition of pup ultrasonic vocalizations by unfamiliar males, heating or maternal contact, and it may be that activation of an endogenous cannabinoid system subserves this role. Future research will hopefully determine whether this is the case.

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