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ORIGINAL INVESTIGATION

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Chronic food restriction in rats augments the central rewarding effect of cocaine and the δ_1 opioid agonist, DPDPE, but not the δ_2 agonist, deltorphin-II

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Abstract *Rationale:* Chronic food restriction augments the self-administration and locomotor stimulating effects of opiates, psychostimulants and NMDA antagonists. The extent to which these effects can be attributed to changes in drug pharmacokinetics and bioavailability versus sensitivity of the neuronal circuits that mediate the affected behavioral functions, has not been established. Recent studies point to central adaptive changes insofar as rewarding, locomotor and c-fos-inducing effects of amphetamine and MK-801, injected directly into the lateral ventricle, are greater in food-restricted than ad libitum fed rats. The increased expression of c-fos in nucleus accumbens (NAC) shell, in particular, suggests that food restriction may augment drug reward by modulating dopamine (DA) synaptic function in this area. *Objectives:* The first purpose of this study was to investigate whether the rewarding effects of cocaine and the δ_1 opioid agonist DPDPE, both of which increase DA synaptic transmission, are augmented by food restriction. The second purpose was to determine whether the δ_2 opioid agonist, deltorphin-II, which has been reported to exert DA-independent rewarding effects, is subject to the potentiating effect of food restriction. *Methods:* Rewarding effects of drugs were measured in terms of their ability to lower the threshold for lateral hypothalamic self-stimulation (LHSS) using a rate-frequency method. *Results:* In separate experiments, cocaine (50, 100 and 150 μg, ICV) and DPDPE (10 and 25 μg, ICV) produced greater threshold-lowering effects in food-restricted than ad libitum fed rats. Deltorphin-II (5.0, 10 and 25 μg, ICV) had no effect on reward thresholds, regardless of feeding regimen. *Conclusions:* While the reported DA-independence of deltorphin-II rewarding effects seemed to offer a means of testing the hypothesis that DA transmission is the critical modulated variable in food-restricted subjects, rewarding effects of this compound could not be demonstrated in the LHSS paradigm. The present results do, however, confirm and extend prior findings indicating that the enhanced self-administration of abused drugs by food-restricted subjects is due to enhanced sensitivity of a final common pathway for drug reward.

Key words Food restriction · Reward · Cocaine · Delta opioid · DPDPE · Deltorphin

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Introduction

Chronic food restriction increases the self-administration and locomotor stimulating effect of opiates, psychostimulants and NMDA antagonists (Campbell and Fibiger 1971; Carroll and Meisch 1984; Deroche et al. 1993; Bell et al. 1997; Cabeza de Vaca and Carr 1998). Because the behavioral studies documenting these effects have invariably involved a systemic route of drug administration, it is not clear whether the apparent increases in behavioral responsiveness result from changes in drug pharmacokinetics and bioavailability, or neuroadaptations that increase sensitivity of neural circuits mediating the affected behavioral functions. Recent evidence supports the latter. First, it was demonstrated that the rewarding effects of systemically administered amphetamine, phencyclidine, and MK-801, indexed by their ability to lower the threshold for electrical brain stimulation reward, are augmented in rats that are food-restricted to 75–80% of initial body weight. Next, this effect, as well as the augmentation of drug-induced locomotion, were shown to be preserved when drugs were administered directly into the lateral cerebral ventricle (ICV) (Cabeza de Vaca and Carr 1998). Most recently, it was shown that food restriction augments ICV amphetamine-induced c-fos expression in several subcortical dopamine (DA) terminal areas (e.g., nucleus accumbens, central nucleus of the amygdala, and bed nucleus of the stria terminalis) (Carr and Kutchukhidze 2000a). In a related study of rats injected ICV with MK-801, only one of these subcortical DA terminal areas, the nucleus acc-

umbens (NAC) shell, displayed an augmentation of drug-induced fos expression in food-restricted subjects (Carr and Kutchukhidze 2000a). In light of evidence that the induction of c-fos by amphetamine and other psychostimulants is mediated by the D_1 receptor (Graybiel et al. 1990; Berretta et al. 1992), and that indirect stimulation of mesocorticolimbic DA transmission by MK-801 accounts, at least in part, for its behavioral effects (Zhang et al. 1992; Criswell et al. 1993; Ouagazzal et al. 1993; Mathe et al. 1998), these results suggest that food restriction augments the behavioral response to abused drugs by modulating DA synaptic function. Moreover, NAC shell, which is critically involved in the rewarding effects of food and drugs (for review see Di Chiara 1999), may be a particularly important locus.

The hypothesis suggested by the findings outlined above predicts that any drug whose rewarding effect depends upon direct or indirect stimulation of DA transmission in NAC shell will benefit from the modulatory effect of food restriction. One purpose of the present study was to determine whether the rewarding effects of cocaine and the δ_1 opioid agonist, DPDPE (Mosberg et al. 1983), administered via the ICV route, are augmented by food restriction. Cocaine blocks the DA transporter (Giros et al. 1996) and its reinforcing effect depends upon DA release in NAC (see McBride et al. 1999). The rewarding effects of δ_1 opioid agonists, such as DPDPE, are well demonstrated (e.g., Shippenberg et al. 1987) and appear to involve sites of action within the ventral tegmental area (Devine and Wise 1994) and NAC (Johnson et al. 1995). Moreover, DPDPE injection in both sites stimulates DA release in NAC (Pentney and Gratton 1991; Devine et al. 1993). The present experiments should therefore assist in defining the scope of the food restriction effect on drug reward and provide additional opportunity to evaluate whether drugs whose rewarding effects appear to involve DA release in NAC necessarily benefit from food restriction.

A second purpose of this study was to determine whether the δ_2 opioid agonist deltorphin-II (Sasaki et al. 1991) exerts a rewarding effect in the electrical brain stimulation reward paradigm, and, if so, whether it is augmented by food restriction. While most rat brain regions contain higher densities of δ_2 than δ_1 receptors (Hiller et al. 1996), very few studies have evaluated the rewarding properties of δ_2 agonists. In mice, it has been shown that ICV deltorphin-II does reinforce a conditioned place preference (Suzuki et al. 1996). In rats, ICV deltorphin-II generalizes to the discriminative stimulus effects of cocaine (Suzuki et al. 1997a). In both studies, deltorphin-II was about 3 times more potent than DPDPE. However, there are two observations suggesting that any rewarding effect of deltorphin-II may not be subject to modulation by food restriction. First, unlike DPDPE, the place preference induced by deltorphin-II is not blocked by pretreatment with the D_1 DA antagonist SCH23390 (Suzuki et al. 1996), and a reinforcing dose does not increase DA turnover in limbic forebrain (Suzuki et al. 1997b). Thus, it has been proposed that δ_2 rewarding effects are mediated by a DA-independent system. Second, persistent negative energy balance produced by streptozotocin augments the analgesic effect of DPDPE but not deltorphin-II (Kamei et al. 1994). Thus, there is precedent for differential regulation of δ_1 and δ_2 receptor mediated behavioral function by persistent negative energy balance.

As in the prior behavioral study (Cabeza de Vaca and Carr 1998), the rewarding effects of drugs were measured in terms of their ability to lower the threshold for lateral hypothalamic self-stimulation (LHSS), using a rate-frequency method. Rate-frequency curves are generated by allowing subjects to lever press for 1-s trains of brain stimulation with stimulation frequency decreasing systematically over a series of trials. A typical curve consists of an asymptotic component (a sequence of high frequencies at which the animal lever presses at its maximal rate) and a descending component (a sequence of descending frequencies at which the animal lever presses at correspondingly decreasing rates). After a regression line is fitted to the descending component of the curve, parameters are derived that have been shown by validation studies (Edmonds and Gallistel 1974; Miliaressis et al. 1986) to be differentially reflective of the rewarding efficacy of stimulation and performance capacity of the subject. The frequency that supports half the maximal reinforcement rate (M-50) is representative of reward efficacy or threshold. Drugs of abuse reliably shift rate-frequency curves to the left, lowering the M-50 (for review, see Wise 1996). The magnitude of this threshold-lowering effect is indicative of the rewarding potency of the drug. The asymptotic or maximal reinforcement rate is reflective of performance capacity. Drugs that raise or lower the asymptote but have little or no effect on M-50 would be interpreted as selectively affecting performance.

Materials and methods

Subjects and surgical procedures

All subjects were 350–400 g male Sprague-Dawley rats housed individually in plastic cages with free access to food and water except when food restriction conditions applied (see below). Animals were maintained on a 12:12-h light:dark cycle, with lights on at 0700 hours and behavioral testing always conducted during the light phase. Experimental procedures were approved by the New York University School of Medicine Institutional Animal Care and Use Committee and were performed in accordance with the "Principles of laboratory animal care" (NIH publication No. 8523, revised 1985).

Several days after arrival in the central animal facility, each rat was anesthetized with ketamine (100 mg/kg, IP) and xylazine (10 mg/kg, IP) and stereotaxically implanted with a 0.25 mm diameter monopolar stimulating electrode (Plastics One, Roanoke, Va., USA) in the lateral hypothalamic medial forebrain bundle (coordinates: 3 mm posterior to bregma, 1.6 mm lateral to the sagittal suture, and 8.6 mm ventral to skull surface). An anterior ipsilateral stainless steel skull screw served as ground. Subjects were also implanted with a 26-gauge guide cannula (Plastics One), containing an occlusion stylet, aimed at the lateral ventricle contralateral to the stimulating electrode (coordinates: 1 mm posterior to

bregma, 1.6 mm lateral to the midline suture, and 3.4 mm ventral to skull surface). The electrode, ground, and cannula were permanently secured to the skull by flowing dental acrylic around them and three additional mounting screws.

Electrical brain stimulation

Brain stimulation training and testing were conducted in four standard test chambers (26×26×21 cm) placed within sound attenuating cubicles. Each chamber had a retractable lever mounted on one wall and a house light mounted on the opposite wall. Four constant current stimulators (PHM-152B/2; Med-Associates, Georgia, Vt., USA) were used to deliver trains of 0.1 ms cathodal pulses, which were conducted to implanted electrodes by way of commutators and flexible cables. Electrical stimulation, contingencies, and data recording were controlled through an IBM PC and interface (Med-Associates). All stimulation parameters were monitored on a Tektronix (TAS 455) oscilloscope.

Self-stimulation training

After 1 week of post-surgical recovery, rats were exposed to the chamber and trained to leverpress for 0.5 s trains of LH stimulation at a frequency of 100 pulses per second (pps). The initial stimulation intensity of 120 μA was systematically manipulated to locate the lowest intensity, for each rat, that would maintain vigorous leverpressing with no signs of aversive or motoric side effects. This initial screening was followed, on subsequent days, by training in a discrete trials procedure. Each training session consisted of twenty-four 60-s trials. Each trial was initiated by extension of the response lever and a 2-s train of "priming" stimulation. Each trial was terminated by retraction of the lever and followed by a 10-s intertrial interval. Each leverpress produced a 1-s train of stimulation, except for those presses emitted during the 1-s train which did not increase reinforcement density. The numbers of leverpresses and reinforcements were recorded for each trial.

Discrete trials training was followed by rate-frequency training, which continued for approximately 2 weeks. Rate-frequency curves were generated by presenting 12 trials in which the frequency of brain stimulation decreased in 0.05 log units over successive trials from an initial frequency of 100 pps to a terminal frequency of 28 pps. Two such series were presented in each training session. During this period, each rat was assigned a stimulation intensity based on its ability to sustain asymptotic responding across the four highest stimulation frequencies in the series. The stimulation intensities thus assigned ranged from 150 to 275 μA.

Food restriction

Once rate-frequency responding had stabilized, rats were paired, based on maximum reinforcement rate and shape of the rate-frequency function, and each member of a pair was randomly assigned to either an "ad libitum" or a "restricted" feeding condition. Rats assigned to the restricted feeding condition received a single 10 g meal (Purina rat chow) each day in the home cage. This represented 40–50% of ad libitum intake in the control group. Rate-frequency testing continued, periodically, over the next 15–20 days until body weights of food-restricted rats had decreased by approximately 20%. During the subsequent drug testing period, feeding of the restricted group was titrated to maintain body weight loss in the range of 20–25%.

Intraventricular microinjection

Drug solutions were loaded into a 30 cm length of PE-50 tubing attached at one end to a 250-μl Hamilton syringe filled with distilled water and at the other end to a 33-gauge injector cannula which extended 1 mm beyond the implanted guide. The syringe was mounted on a Harvard 2272 microliter syringe pump which delivered the 5 μl injection volume over a period of 95 s. One minute following injections, injector cannulae were removed, stylets replaced, and animals were returned to the test chambers where post-injection tests were initiated 10 m after completion of the ICV injection. The accuracy of cannula placements had been verified two weeks prior to drug testing by demonstrating a vigorous and short latency (i.e., <60 s) drinking response to 50 ng angiotensin II.

Drugs and dose-response testing

During drug testing, each session began with a pre-injection test consisting of three rate-frequency series. The first series was considered to be a "warm-up" and data were not included in the calculation of pre-injection LHSS parameters. Injection of drug or saline vehicle was followed by a post-injection test consisting of two rate-frequency series. A complete dose-response study consisted of three or four sessions, spaced at least 48 h apart. For each dose-response study, the order in which vehicle and drug doses were administered was counterbalanced across sessions and subjects with daily treatments matched across groups.

The first two groups of nine food-restricted and ten ad libitum fed rats were tested as to the effects of ICV cocaine HCL (National Institute on Drug Abuse). By the first day of drug testing the mean body weight of food-restricted rats had declined from 451 (±17.9) to 357 (±14.8) g and body weight of ad libitum fed controls had increased from 457 (±15.4) to 480 (±13.0) g. Cocaine was administered in doses of 0.0, 50.0, 100.0, and 150 μg (ICV).

Two new groups of seven food-restricted and seven ad libitum fed rats were used to test the effect on LHSS of 0.0, 10.0, and 25 μg doses of DPDPE (H-Tyr-D-Pen-Gly-Phe-D-Pen-OH; NIDA via Multiple Peptide Systems, San Diego, Calif., USA) and, after 1 week of recovery, 0.0, 5 and 10 μg doses of deltorphin-II (Tyr-D-A;a-Phe-Glu-Val-Val-Gly-NH_2; Research Biochemicals, Natick, Mass., USA). On the first day of testing with DPDPE body weights of food-restricted rats had declined from 403 (±7.7) to 315 (±5.4) and weights of ad libitum fed rats had increased from 406 (±11.1) to 430 (±11.3) g.

Several days after the completion of testing with deltorphin-II all rats were tested as to the effect of d-amphetamine (0.5 mg/kg, IP, 10 min prior to testing) to verify that the food-restricted group continued to express an augmented threshold-lowering effect in response to a classical psychostimulant.

Finally, in order to verify that the lack of effect of deltorphin-II was not due to use of insufficient doses, a 25 μg dose was tested.

Data analysis

The average pre- and post-injection rate-frequency curves obtained for each rat per session were used to derive two LHSS parameters that can be used to distinguish between changes in reward efficacy and performance capacity (Edmonds and Gallistel 1974; Miliaressis et al. 1986). The asymptotic (or maximum) reinforcement rate, which is reflective of performance capacity, was described by a line that paralleled the *x*-axis and was defined as the mean of all consecutive values within 10% of the highest rate for the curve. All remaining values formed the descending portion of the curve, with the lowest point being at the highest frequency to produce fewer than 2.5 reinforcements per minute. Regression analysis of the descending portion of the curve was used to calculate the M-50 measure of reward threshold defined as the log pulse frequency sustaining half the maximum reinforcement rate. Following calculation of reward thresholds, antilog transformations were applied and natural frequencies were used to calculate the percentage changes occurring in the post-injection test relative to the pre-injection test.

For each parameter, treatment effects were evaluated by two-way mixed analysis of variance (ANOVA) with the drug dose (3 or 4) as a within-subjects factor and feeding regimen (2) as a be-

tween-subjects factor (Systat software). Since the experimental hypothesis and prior work predicted a greater threshold-lowering effect of drugs in food-restricted rats, planned unidirectional comparisons between groups at each dose level were performed using the pooled error term from the ANOVA in the denominator of a *t*-statistic.

Histology

Upon the completion of behavioral testing, rats were overdosed with sodium pentobarbital (100 mg/kg, IP) and brains were removed. After a minimum of 48 h in 10% buffered formalin, frozen coronal sections, 30 μm thick, were cut on an IEC Minotome and stained with cresyl violet.

Results

Cocaine lowered the reward threshold and had a significantly greater effect in food-restricted as compared to ad libitum fed subjects [Fig. 1; $F_{cocaine}(3,51)=19.7$, $P<0.0001$; $F_{diet}(1,17)=13.2$, $P<0.005$; $F_{cocaine\times diet}(3,51)=2.8$, $P=0.05$]. Comparisons between groups at each cocaine dose level confirmed a greater threshold-lowering effect in food-restricted rats at the 50 μg [$t(17)=2.33$, $P<0.025$], 100 μg [$t(17)=2.04$, $P<0.05$] and 150 μg dose [$t(17)=4.02$, $P<0.0005$]. There were no significant main effects of cocaine, diet, nor any interaction between these factors in relation to maximum reinforcement rate [$F_{cocaine}(3,51)=2.0$; $F_{diet}(1,17)=0.0$; $F_{cocaine\times diet}(3,51)=0.2$.] Illustrative rate-frequency curves, representing the effects of the 150 μg dose of cocaine, are displayed in Fig. 2.

The δ_1 opioid agonist, DPDPE, also lowered the reward threshold with a greater effect in food-restricted as compared to ad libitum fed rats [Fig. 3; $F_{dpdpe}(1,24)=12.6$, $P<0.001$; $F_{diet}(1,12)=6.5$, $P<0.025$; $F_{dpdpe\times diet}(2,24)=4.3$, $P<0.025$]. Comparison between groups at each DPDPE dose level confirmed a greater threshold-lowering effect in food-restricted rats following the 25 μg dose [$t(12)=3.7$, $P<0.005$]. The maximum reinforcement rate was not affected by DPDPE, diet or interaction between these factors [$F_{dpdpe}(2,24)=1.2$; $F_{diet}(1,12)=1.5$; $F_{dpdpe\times diet}(1,12)=2.1$).

The δ_2 opioid agonist, deltorphin-II, had no effect on reward threshold, nor was there an interaction between

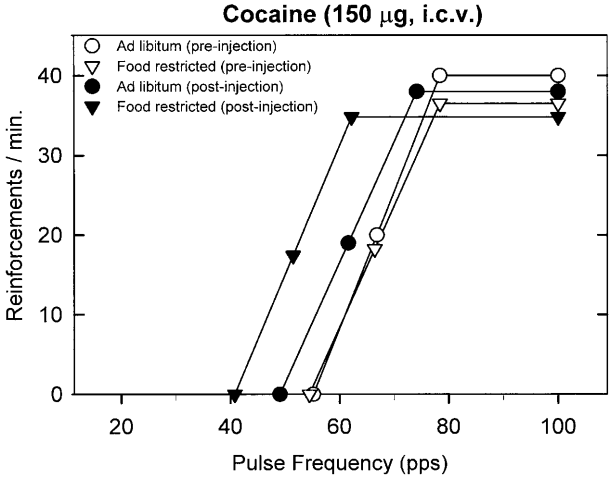

Fig. 2 Illustrative rate-frequency curves, representing the effect of cocaine (150 μg, ICV) on self-stimulation in ad libitum fed and food-restricted rats. The curves displayed are based on the mean maximum reinforcement rates, M-50 reward thresholds, and *x*-axis intercepts of subjects in pre- and post-injection tests

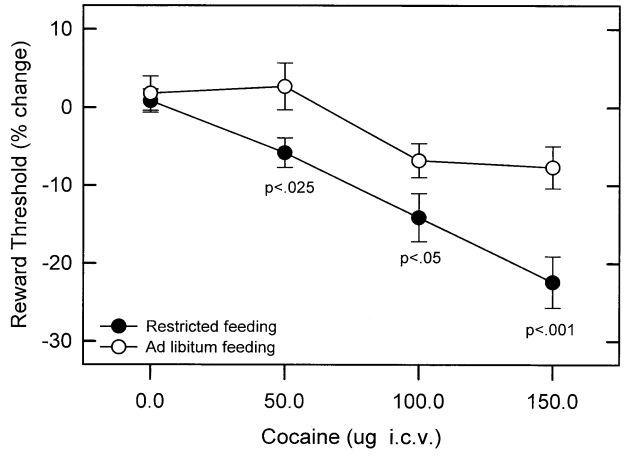

Fig. 1 Mean (±SEM) percentage change in reward threshold as a function of cocaine dose for food-restricted (*filled circles*) and ad libitum fed (*open circles*) rats. Reward thresholds were derived from LHSS rate frequency curves obtained immediately before and 10 min after intracerebroventricular administration of cocaine. The mean reward thresholds of food-restricted and ad libitum fed rats obtained in the test that preceded vehicle injection (i.e., dose 0 μg) were 66.6±2.5 and 67.1±3.6 pulses per second, respectively. *P*-values refer to differences between groups at the indicated dose levels based on *t*-tests that followed analysis of variance

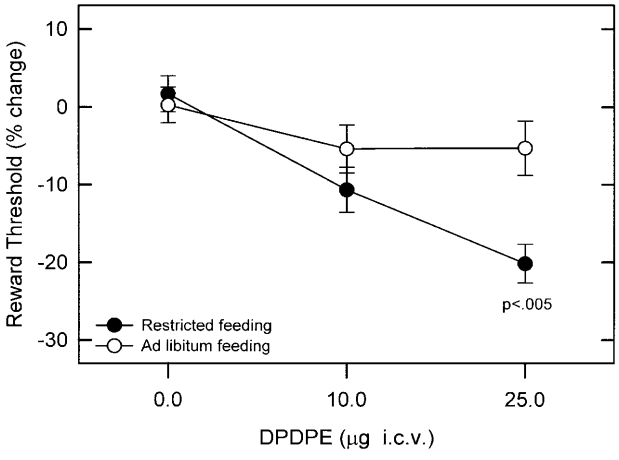

Fig. 3 Mean (±SEM) percentage change in reward threshold as a function of DPDPE dose for food-restricted (*filled circles*) and ad libitum fed (*open circles*) rats. Reward thresholds were derived from LHSS rate frequency curves obtained immediately before and 10 min after intracerebroventricular administration of DPDPE. The mean reward thresholds of food-restricted and ad libitum fed rats obtained in the test that preceded vehicle injection (i.e., dose 0 μg) were 68.3±3 and 63.3±2.7 pulses per second, respectively. *P*-value refers to the difference between groups at the indicated dose level based on *t*-tests that followed analysis of variance

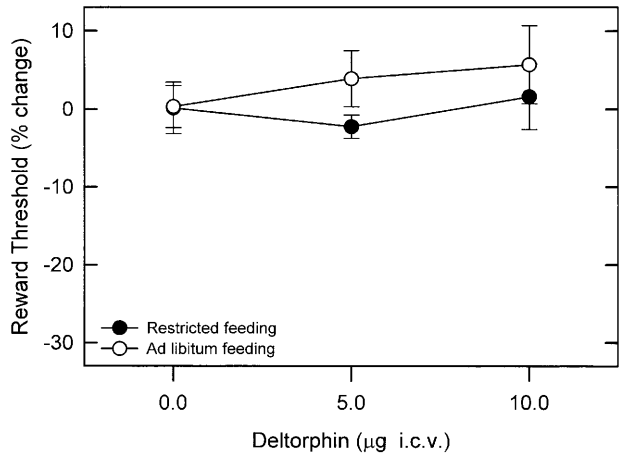

Fig. 4 Mean (±SEM) percentage change in reward threshold as a function of deltorphin-II dose for food-restricted (*filled circles*) and ad libitum fed (*open circles*) rats. Reward thresholds were derived from LHSS rate frequency curves obtained immediately before and 10 min after intracerebroventricular administration of deltorphin-II

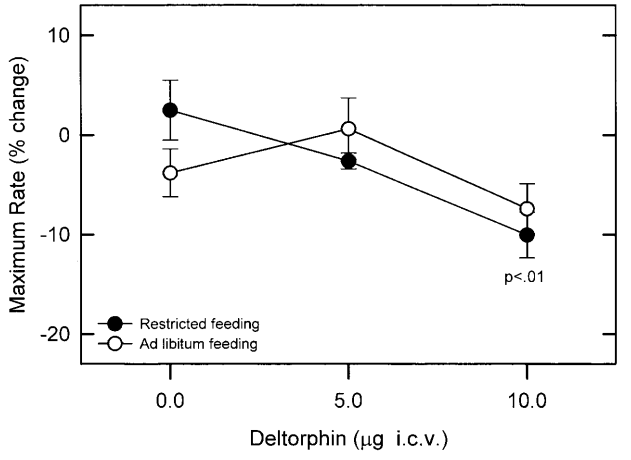

Fig. 5 Mean (±SEM) percentage change in maximum reinforcement rate as a function of deltorphin-II dose for food-restricted (*filled circles*) and ad libitum fed (*open circles*) rats. Maximum reinforcement rates were derived from LHSS rate frequency curves obtained immediately before and 10 min after intracerebroventricular administration of deltorphin. The mean maximum reinforcement rates of food-restricted and ad libitum fed rats obtained in the test that preceded vehicle injection (i.e., dose 0 μg) were 39.7±1.6 and 40.2±1.3 reinforcements per minute, respectively. *P*-value refers to the difference between dose levels, across feeding groups, based on the analysis of variance

diet and deltorphin-II [Fig. 4; $F_{\text{deltorphin}}(2,24)=0.7$; $F_{\text{diet}}(1,12)=0.9$; $F_{\text{deltorphin}\times\text{diet}}(2,24)=0.5$]. Deltorphin did, however, decrease the maximum reinforcement rate with no differential effect of diet [Fig. 5; $F_{\text{deltorphin}}(2,24)=6.2$, $P<0.01$; $F_{\text{diet}}(1,12)=0$; $F_{\text{deltorphin}\times\text{diet}}(2,24)=2.1$].

Following the completion of deltorphin testing, amphetamine challenge in these same rats produced the typical lowering of reward threshold with a significantly greater effect in the food-restricted group [Fig. 6; $t(10)=2.69$, $P<0.02$].

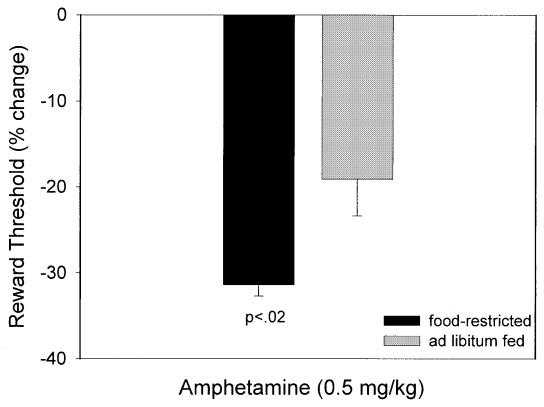

Fig. 6 Mean (±SEM) percentage change in reward threshold produced by amphetamine in ad libitum fed (*light bar*) and food-restricted (*dark bar*) rats. Reward thresholds were derived from LHSS rate frequency curves obtained immediately before and 10 min after intraperitoneal injection of amphetamine

In a final test, to ensure that the lack of effect of deltorphin-II was not due to the use of insufficient doses, five ad libitum fed and six food-restricted rats were tested with a 25 μg dose of deltorphin-II. Again, this compound failed to affect reward thresholds, producing elevations of 3.08% (±4.0) and 1.35% (±6.8) in ad libitum fed and food-restricted rats, respectively. As observed in response to the 10 μg dose, deltorphin again decreased the maximum reinforcement rate by –7.7% (±3.9) and –10.7% (±2.7), in ad libitum fed and food-restricted rats, respectively.

Discussion

Food-restricted animals self-administer more cocaine and opiates, and respond for lower reinforcing doses, than do ad libitum fed subjects (for review, see Carroll and Meisch 1984). The present results attribute these observations, at least in part, to enhanced central sensitivity to rewarding effects of these drugs. It has now been demonstrated that rewarding effects of centrally administered amphetamine and MK-801 (Cabeza de Vaca and Carr 1998), cocaine and DPDPE are augmented by chronic food restriction. Insofar as these drugs are representative of several different chemical classes and different primary mechanisms of neuropharmacologic action, it would seem that food restriction augments responsiveness of a final common pathway for drug reward. The DA synapse in NAC, particularly the shell, has been proposed as a common synapse in the mediation of diverse drug rewarding effects (Pontieri et al. 1995; Carlezon and Wise 1996), and augmentation of cellular responses in this region to centrally administered drugs has been demonstrated in recent c-fos immunohistochemical investigations (Carr and Kutchukhidze 2000a, 2000b).

Intravenous cocaine preferentially increases DA release in NAC shell (Pontieri et al. 1995) and although early intracerebral self-administration studies indicated

that medial prefrontal cortex (mPFC) and not NAC supports self-administration responding (Goeders and Smith 1993), self-administration in mPFC was shown secondarily to increase DA release in NAC (Goeders and Smith 1993). Moreover, recent studies have succeeded in demonstrating cocaine self-administration in NAC (see McBride et al. 1999). The importance of this DA synapse in cocaine rewarding effects is underscored by demonstration that 6-hydroxydopamine lesions of NAC block cocaine self-administration (Pettit et al. 1984).

Evidence that the δ antagonist, naltrindole, blocks the rewarding effect of cocaine (Reid et al. 1993, 1995) raises the possibility that δ opioid rewarding effects may be mediated downstream from the DA synapse. Indeed, about 35% of the δ receptors in NAC are located postsynaptically on dendrites of the medium spiny output neurons (Svingos et al. 1998) and 6-OHDA lesions of NAC do not diminish the locomotor response to locally injected DPDPE (Churchill and Kalivas 1992). If the rewarding effect of DPDPE could be placed anatomically downstream or in parallel with the DA synapse, that would have important implications for the effort to localize and characterize the mechanism through which food restriction augments the rewarding effect of abused drugs. However, DPDPE microinjection in the ventral tegmental area has not only been shown to be rewarding (Devine and Wise 1994), but stimulates DA release in NAC (Devine et al. 1993). In addition, microinjection of DPDPE in NAC is rewarding (Johnson et al. 1995) and augments impulse-dependent DA release from terminal endings within the NAC (Pentney and Gratton 1991). Finally, DA antagonists have blocked the rewarding effect of DPDPE (Duvauchelle et al. 1997). Thus, DA release in NAC is stimulated by all drugs that have so far proved vulnerable to the potentiating effect of food restriction. Augmentation of DA synaptic function in NAC therefore remains a tenable hypothesis to account for the effect of chronic food restriction.

Deltorphin-II represented a possible means of challenging the aforementioned hypothesis because conditioned place preference experiments in mice indicated that this δ_2 opioid agonist has rewarding properties that do not depend on DA transmission (Suzuki et al. 1996, 1997b). However, this compound displayed no rewarding properties in the electrical brain stimulation reward paradigm. This is not likely to result from use of insufficient dosage because deltorphin-II is several times more potent than DPDPE in producing other behavioral effects (Suzuki et al. 1997a, 1997b). Moreover, deltorphin-II was not without effect in the current paradigm. It produced a small but significant decrease in maximum reinforcement rate in both ad libitum fed and food-restricted subjects. This suggests a small effect on performance capacity. There are drugs, with demonstrated rewarding properties, that do not lower the brain stimulation reward threshold. For example, direct DA receptor agonists, that are self-administered, tend to raise the threshold for brain stimulation reward. In some cases, this is clearly due to the presence of competing stereotypies (Hall and Stellar 1996). In other cases it has been postulated that non-contingent stimulation of DA receptors masks the effect of stimulation-induced DA release and uncouples receptor stimulation from the instrumental response, thereby diminishing reinforcement of lever pressing (Beninger and Miller 1998; Baldo et al. 1999). The absence of effect of deltorphin-II on reward thresholds, and the absence of discernable unconditioned behavioral responses, however, casts doubt on alternatives to the conclusion that the δ_2 opioid receptor does not mediate rewarding effects in the self-stimulation paradigm.

It has been postulated that the neuronal circuitry that mediates rewarding effects of abused drugs is normally involved in mediating adaptive behavior, impelling animals toward and reinforcing contact with goal objects that satisfy active survival needs (Wise 1982; Di Chiara and North 1992; Koob 1992). The association between feeding and drug reward has been most extensively studied and substantiated. In addition to the potentiating effect of food restriction on drug reward, it has been shown that the self-administration and locomotor responses to morphine (Gosnell et al. 1995) and amphetamine (Sills and Vaccarino 1994), respectively, can be predicted from the propensity of animals to ingest sweet solutions. Moreover, cocaine self-administration can be attenuated by providing concurrent access to sweet solutions (Comer et al. 1996). At the level of the NAC, an intimate association between food and drug reward is suggested as well. While microinjection of amphetamine (Vaccarino 1994) and opiates (Zhang and Kelley 1997) in the NAC will elicit or augment ingestive behavior, animals will self-inject these compounds into the NAC in the absence of food (Hoebel et al. 1983; Goeders et al. 1984; Phillips et al. 1994). Furthermore, contact with a novel and/or palatable food preferentially stimulates DA release in the NAC shell (Wilson et al. 1995; Bassareo and Di Chiara 1999) as does intravenous administration of diverse drugs of abuse (Pontieri et al. 1995). In addition, the propensity of animals to ingest sugar predicts the magnitude of their NAC DA response to amphetamine (Sills and Crawley 1996). Importantly, food deprivation augments the DA releasing effect of food in NAC (Wilson et al. 1995) and food restriction augments the DA releasing effect of locally injected amphetamine (Pothos et al. 1995). It therefore seems likely that the augmentation of drug reward by food restriction results from an adaptive modification of NAC DA synaptic function that normally invigorates the behavioral response to food stimuli.

The physiological signals antecedent to this modification are not known. However, the type of persistent neuroadaptations that underlie sensitization of drug-induced locomotion following chronic intermittent stress (Kalivas and Stewart 1991) or drug regimens (Pierce and Kalivas 1997) are not likely to be involved. It has recently been observed that the augmented locomotor response to amphetamine in food-restricted rats is reversed within several days of restored ad libitum access to food (Cabeza de Vaca and Carr, unpublished). Thus, it would appear

that the enhanced central sensitivity is due to the presence of a dynamic signal that is rapidly responsive to changes in the underlying condition. Recent discoveries of multiple hormones and neuropeptides that respond to changes in body weight status and energy balance, and consequently regulate ingestive behavior, suggest numerous candidate signals. Leptin and insulin levels, both of which decline substantially in food-restricted rats, have recently been shown to modulate brain reward circuitry (Carr et al. 2000; Fulton et al. 2000). The MC-4 receptor, through which α-MSH suppresses and agouti-related protein increases food intake, is not only present in ventral tegmental area and NAC shell but shows similar adaptive changes in obese and opiate-treated animals (see Lindblom et al. 2000). Another system that may be important consists of the CART peptides. CART mRNA levels are regulated by leptin as well as by cocaine and amphetamine, and both the peptides and mRNA have been localized to NAC and ventral tegmentum (for review see Kuhar and Dall Vechia 1999).

In conclusion, the present results confirm and extend prior findings that chronic food restriction enhances central sensitivity to the rewarding effects of abused drugs. While immunohistochemical findings, and the mechanisms of action of the drugs studied, suggest that augmentation of DA synaptic transmission in NAC shell may underlie this phenomenon, future research may definitively establish the synaptic mechanisms through which the modulatory effect of food restriction is exerted. An additional, and crucial challenge, is identification of the particular cascade of signals associated with chronic food restriction that leads to enhanced central sensitivity to the rewarding effects of abused drugs.

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