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Ingestive Behavior Following Central [D-Ala^2,Leu^5,Cys^6]-Enkephalin (DALCE), a Short-Acting Agonist and Long-Acting Antagonist at the Delta Opioid Receptor

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ARJUNE, D., W. D. BOWEN AND R. J. BODNAR. *Ingestive behavior following central [D-Ala^2,Leu^5,Cys^6]-enkephalin (DALCE), a short-acting agonist and long-acting antagonist at the delta opioid receptor.* PHARMACOL BIOCHEM BEHAV **39**(2) 429–436, 1991.—DALCE (1–40 μg, ICV), a short-acting agonist and long-acting antagonist at the delta opioid receptor, was examined for its effects upon food intake in rats under spontaneous, deprivation, glucoprivic and palatable conditions. DALCE (10 μg) significantly stimulated free feeding for up to 10 h but only minimally decreased (40 μg) food intake and body weight after 24–72 h. DALCE, administered prior to food deprivation (24 h), failed to affect subsequent 24-h intake and sporadically decreased intake and body weight change after 48–72 h. 2-Deoxy-D-glucose (650 mg/kg, IP) hyperphagia was transiently (2 h) decreased by long-term DALCE (10 μg) pretreatment. Hyperphagia following exposure to a high-fat diet was significantly potentiated by long-term DALCE (1 μg) pretreatment. DALCE (10 μg) hyperphagia (2–10 h) was eliminated by central pretreatment with either naltrexone (20 μg) or the kappa antagonist, nor-binaltorphamine (20 μg) but was minimally affected by central pretreatment with the mu antagonist, beta-funaltrexamine (20 μg) or long-term DALCE (40 μg). The general inability of the antagonist actions of DALCE to alter these forms of feeding argues against a role for the delta opioid receptor in these responses.

[D-Ala^2,Leu^5,Cys^6]-enkephalin (DALCE) Free feeding Deprivation feeding 2-Deoxy-D-glucose feeding High-fat feeding Delta opioid receptor Rats

THE development of highly selective antagonists for different opiate receptor subtypes (29,35) has allowed for a more precise analysis of each subtype's role in the opioid mediation of different forms of ingestive behavior [see reviews: (26,40)]. The general opiate antagonists, naloxone and naltrexone, suppress food intake following deprivation (5, 9, 14, 17), 2-deoxy-D-glucose (2DG) glucoprivation (2,30), opiate agonist treatment (26,40) and introduction of palatable diets (1, 2, 10, 12, 18, 32). Beta-funaltrexamine (B-FNA), a short-acting kappa agonist and irreversible mu antagonist (42, 50, 53, 54) initially stimulates free feeding, which is blocked by the selective kappa antagonist, nor-binaltorphamine [Nor-BNI: (43,49)] but not by its own long-acting mu-antagonist properties (3,52). Reductions occur in free and deprivation feeding as well as hyperphagia following 2DG and exposure to a high-fat diet by central B-FNA (3, 18, 24, 52), implicating the mu receptor. Naloxonazine, an irreversible mu_1 antagonist [e.g., (41)], decreases free and deprivation feeding as well as morphine hyperphagia (33, 34, 46) but fails to alter hyperphagia following 2DG (46), exposure to a high-fat diet (18) or administration of dynorphin A, [D-Ala^2,D-Leu^5]-enkephalin (DADL) and ethylketocyclazocine (33). The kappa antagonist, Nor-BNI, decreases nocturnal and deprivation intake as well as hyperphagia following opiate agonists, glucoprivation and exposure to a high-fat diet (2,23).

Mediation of food intake by the delta opioid receptor was based upon the stimulatory actions of such delta-selective agonists as DADL, [D-Ser^2,Leu^5]-enkephalin-Thr^6 (DSLET), and [D-Ala^2]-Met-enkephalinamide (DALA) (15, 16, 31, 48, 51), and the inhibitory actions of ICI 174,864, a delta-selective antagonist (13) upon free feeding and DADL hyperphagia (21). Since DSLET hyperphagia is eliminated by Nor-BNI (23), one cannot make definitive statements regarding opioid receptor subtype mediation using agonists alone. Further, ICI 174,864 is short acting (13) and degrades into a peptide with mu agonist activity (8). Moreover, the hypophagic properties of ICI 174,864 might be due to motor dysfunction, since it suppressed high-fat intake only at doses that produced motor dysfunction (18). Intrathecal administration of ICI 174,864 also produces hindlimb motor dysfunction and persistent paraplegia (28), suggesting that evidence implicating the delta receptor in feeding behavior is not

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as clear-cut as that for other opiate receptor subtypes.

DALCE is a short-acting delta, and secondarily mu, agonist since it binds delta receptors with high affinity, mu receptors with moderate affinity and kappa receptors with low affinity (4). DALCE dissociates readily from mu receptors but fails to dissociate from delta receptors, forming a covalent attachment by a disulfide bond with a receptor sulfhydryl group. Hence long-lasting and selective delta antagonism subsequently develops, presumably due to receptor desensitization, which has been confirmed in analgesia studies (6, 7, 22). DALCE increased hot-plate latencies shortly after administration which were blocked by naloxone or the delta antagonist, M80 (7). In contrast, long-term (24–72 h) DALCE pretreatment blocked analgesia induced by the delta-selective agonist, [D-Pen^2,D-Pen^5]-enkephalin, but not analgesia induced by the selective kappa agonist, U50,488H, or the selective mu agonist, [D-Ala^2,N-Me^4,Gly^5-ol]-enkephalin (DAMGO) (6, 7, 22).

The present study examined the dose-dependent alterations in food intake following intracerebroventricular (ICV) administration of DALCE in five situations: a) short-term (2–10 h) and long-term (24–72 h) pretreatment with DALCE upon intake in freely feeding rats; b) long-term (24–72 h) pretreatment with DALCE upon hyperphagia following 24 h of food deprivation; c) long-term (24 h) pretreatment with DALCE upon glucoprivic hyperphagia induced by 2DG (47); d) long-term (24 h) pretreatment with DALCE upon hyperphagia induced by exposure to a palatable high-fat diet; and e) effects upon short-term (2–10 h) DALCE hyperphagia following pretreatment with either the general opioid antagonist, naltrexone, the kappa antagonist, Nor-BNI, the mu antagonist, B-FNA, or the long-term delta antagonism of DALCE.

METHOD

Subjects and Surgery

Adult male albino Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, 80–120 days of age) were housed individually in wire-mesh cages and maintained on a 12-h light: 12-h dark cycle with Purina rat chow and water available ad lib. Each rat was pretreated with chlorpromazine (3 mg/kg, IP) and anesthetized with ketamine HCl (100 mg/kg, IM). A stainless steel guide cannula (22 gauge, Plastic Products) was placed stereotaxically (Kopf Instruments) 0.3 mm above the left lateral ventricle by using the following coordinates: incisor bar (+5 mm), 0.5 mm anterior to the bregma suture, 1.3 mm lateral to the sagittal suture and 3.6 mm from the top of the skull. The cannula was secured to the skull by three anchor screws with dental acrylic. All animals were allowed at least one week to recover from stereotaxic surgery before behavioral testing began to allow full drug clearance. All animals were screened for a three-day period before and after surgery for food intake and body weight. Only animals that displayed normal food intake (range: 18–27 g) and body weight gain (2–4 g/day) were used in experimental procedures; approximately 3% of animals recovering from surgery failed to meet this criteria. At the completion of testing, all animals were sacrificed using an overdose of anesthesia (Euthanasia, H. Schein). Cannula placements were verified by visual inspection; all animals included in the present studies had proper cannula placements.

DALCE and Free Feeding

Cannulated rats received between two and four microinjection conditions at weekly intervals; the first microinjection condition always served as a vehicle (n=22) injection. In the subsequent microinjection conditions, rats received in counterbalanced order one or more of the following doses of DALCE: 1 (n=7), 10 (n=7), 20 (n=16) and 40 (n=11) μg. DALCE was synthesized by Peninsula Laboratories using solid-phase techniques. The crude material was purified by thin-layer chromatography, and its purity was checked by reverse-phase HPLC (4). Preweighed aliquots of DALCE were prepared fresh on the day of use and dissolved with 0.2 M hydrochloric acid. The pH was raised to 7.5–8.0 by adding 0.2 M sodium hydroxide, and serial dilution of the stock solution yielded the above doses. Sterile water adjusted to a similar pH was used as the vehicle solution. All ICV infusions were administered in 10-μl volumes at a rate of 1 μl every 10 s through a stainless steel internal cannula (28 gauge, Plastic Products) that protruded 0.5 mm past the tip of the guide cannula, which was connected to a Hamilton microsyringe by polyethylene tubing. All injections took place at the beginning of the light cycle, and food intake was measured at 2, 4, 6, 10, 24, 48, 72 and 96 h after each injection. Intake in this and all but the high-fat protocols was determined by weighing food pellets prior to and after each condition with adjustments for spillage, which was collected by paper under the wire-mesh cage. Body weight was assessed prior to, and at 24, 48, 72 and 96 h after, each injection. Animals in this and subsequent protocols received additional injections only when they had reachieved the baseline body weights prior to the last injection.

DALCE and Deprivation-Induced Feeding

Additional cannulated rats received between two and three microinjection conditions at weekly intervals: vehicle (n=18), and, in counterbalanced order, DALCE at doses of 1 (n=8), 10 (n=8), 20 (n=18) and 40 (n=9) μg. All injections took place between 1 and 2 h into the light cycle, and food was removed after each injection. A twenty-four h period elapsed to allow for development of irreversible antagonism of delta receptors (4, 6, 7, 22) as well as development of food deprivation. Food was then reintroduced, and intake was measured at 2, 4, 24, 48 and 72 h thereafter. Body weight was assessed immediately prior to, and at 24, 48 and 72 h after, injection.

DALCE and Glucoprivic Feeding

Additional cannulated rats received between three and four conditions at weekly intervals: a) vehicle (10 μl, ICV)/vehicle (1.5 ml normal saline/kg body weight, IP) (n=15); b) vehicle/2DG (650 mg/kg, IP, Sigma) (n=15); c) DALCE (1 μg, ICV)/2DG (n=11); d) DALCE (10 μg, ICV)/2DG (n=12); and e) DALCE (20 μg, ICV)/2DG (n=15). Twenty-four h elapsed between the central and peripheral injections, which occurred 1–2 h into the light cycle. Food intake was measured at 2, 4, 6 and 24 h after the peripheral injection.

DALCE And High-Fat Feeding

Additional cannulated rats were adapted to the placement of a preweighed high-fat diet (67% ground laboratory chow +33% vegetable shortening; 5.5 kcal/g: 11.3% protein, 61.3% fat, 27.4% carbohydrate) (18) in their cages for 2 h, beginning 4–6 h into the light cycle. When rats displayed stable intakes of the high-fat diet in the absence of injections, they received between three and four microinjection conditions 24 h prior to testing at weekly intervals: a) vehicle (10 μl, ICV) (n=12) and, in counterbalanced order, DALCE at doses of b) 1 (n=7), c) 10 (n=11) and d) 20 (n=11) μg. Intake, adjusted for spillage, was monitored 1 and 2 h after introduction, and then the diet was

removed from the cage. A noninjection condition was interspersed 120 h after each injection condition to confirm a return of baseline feeding.

Antagonist Effects on Short-Term DALCE Feeding

Additional cannulated rats received between three and five conditions at weekly intervals: a) vehicle (10 μl, ICV)/vehicle (10 μl, ICV) (n=26); b) vehicle/DALCE (10 μg, ICV) (n=26); c) Nor-BNI (20 μg, ICV, Research Biochemicals Inc.)/DALCE (n=12); d) naltrexone (20 μg, ICV, Sigma)/DALCE (n=13); e) B-FNA (20 μg, ICV, Research Biochemicals Inc.)/DALCE (n=12); and f) DALCE (40 μg, ICV)/DALCE (n=13). A 1-h interval elapsed between injections in the first four conditions for peak antagonism of all opiate receptors for naltrexone (45,58) and of kappa receptors for Nor-BNI (49). The 24-h interval in the last two conditions allowed for irreversible blockade of mu receptors for B-FNA (50, 53, 54) and of delta receptors for DALCE (4, 6, 7, 22). All injections occurred 1–2 h into the light cycle, and food intake was measured at 2, 4, 6 and 10 h after the second microinjection.

Statistical Analyses

Analyses of variance were performed to ascertain significant effects among conditions for specific time points in all studies with Dunnett comparisons ($p<0.05$) used to discern significant, individual effects between vehicle and treatment conditions. In the antagonist/DALCE feeding study, Dunn comparisons ($p<0.05$) were used to discern differences between vehicle/DALCE and antagonist/DALCE conditions.

RESULTS

DALCE and Free Feeding

Significant differences in food intake were observed among vehicle and DALCE dose conditions at 4 h, $F(4,58)=3.53$, $p<0.012$, 6 h ($F=4.52$, $p<0.003$) and 10 h ($F=3.67$, $p<0.0099$) but not at 2 h ($F=1.93$) after injection. The upper panel of Fig. 1 indicates the significant increases in free feeding observed following the 10-μg dose of DALCE at 4 (77%), 6 (69%) and 10 (46%) h after injection. Thus DALCE displays a short-term stimulatory effect upon free feeding following central administration which does not appear to follow a monotonic dose-response function.

Significant differences in food intake failed to occur among vehicle and DALCE dose conditions at 24 h, $F(4,58)=1.06$, 48 h ($F=0.46$), 72 h ($F=1.61$) and 96 h ($F=1.18$) after injection. The lower panel of Fig. 1 indicates the levels of free feeding following vehicle and DALCE. With the exception of a transient and nonsignificant decrease in free feeding 24 h following administration of the highest DALCE dose (20%), DALCE failed to alter free feeding at these intervals when it irreversibly antagonizes delta receptors (4, 6, 7, 22).

Significant changes in body weights during free feeding were observed among vehicle and DALCE conditions, $F(4,58)=7.64$, $p<0.0001$, and across the time course, $F(4,232)=23.27$, $p<0.0001$. Vehicle-treated rats failed to display changes in body weight at 24 and 48 h after injection, and significantly increased body weight at 72 and 96 h after injection (Table 1). In contrast, DALCE produced a significant and dose-dependent reduction in body weight at 24 h after injection and suppressed the subsequent body-weight gain observed in vehicle-treated rats. To evaluate whether DALCE was altering feeding efficiency, a ratio of body weight/food intake was determined across dose and

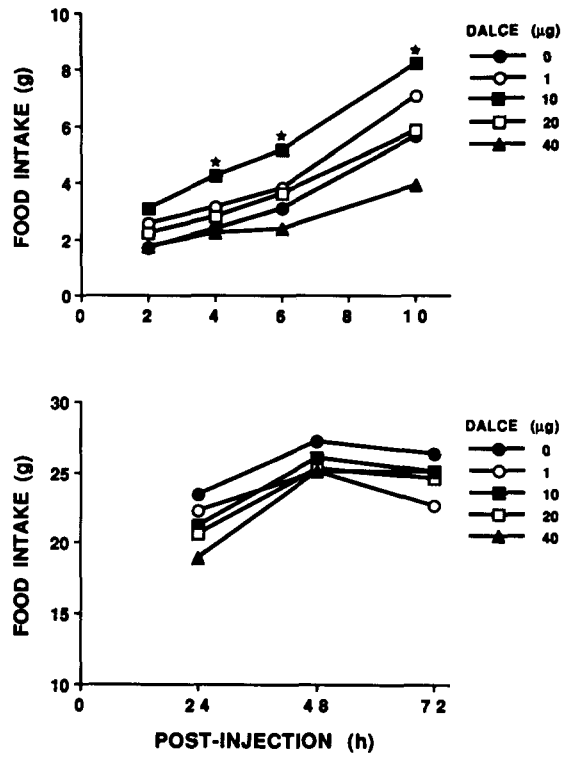

FIG. 1. Alterations in food intake (g) following intracerebroventricular (ICV) administration of vehicle (n=22) or [D-Ala^2,Leu^5,Cys^6]-enkephalin (DALCE) at doses of 1 (n=7), 10 (n=7), 20 (n=16) or 40 (n=11) μg after 2–10 (upper panel) and 24–72 h (lower panel). The stars in this and all subsequent figures denote significant differences in food intake following given doses of DALCE relative to vehicle at each corresponding time point (Dunnett comparisons, $p<0.05$). The following are ranges of the standard errors of the mean (SEM) across conditions at 2 (0.2–0.7 g), 4 (0.3–0.6 g), 6 (0.3–0.9 g), 10 (0.5–1.6 g), 24 (0.5–3.3 g), 48 (0.6–2.2 g) and 72 h (0.7–1.6 g).

time conditions. Significant differences in feeding efficiency were observed across the time course, $F(3,174)=5.63$, $p<0.001$, and for the interaction between conditions and times, $F(12,174)=2.21$, $p<0.013$, but not among conditions, $F(4,58)=1.65$. Significant changes in feeding efficiency only occurred at 24 h following the 40-μg dose of DALCE.

DALCE and Deprivation-Induced Feeding

DALCE failed to produce significant differences in deprivation-induced feeding at 2 h, $F(4,56)=0.61$, 4 h ($F=0.31$) and 24 h ($F=0.52$) after reintroduction of food (Fig. 2, upper and lower panels) but significantly reduced deprivation-induced feeding at 48 h ($F=2.75$, $p<0.037$) and 72 h ($F=5.07$, $p<0.002$) after reintroduction of food (Fig. 2, lower panel). The significant long-term decreases in deprivation-induced intake following DALCE were not dose-dependent in that inhibition occurred only following the 1-μg (48 h: 15%) and 10-μg (48 h: 13%; 72 h: 15%) doses of DALCE.

Significant changes in deprivation-induced body weight were observed across the time course, $F(4,224)=284.94$, $p<0.0001$, and for the interaction between conditions and times, $F(16,224)=2.03$, $p<0.013$, but not among conditions, $F(4,56)=2.13$. DALCE failed to alter the weight loss incurred following 24 h of food

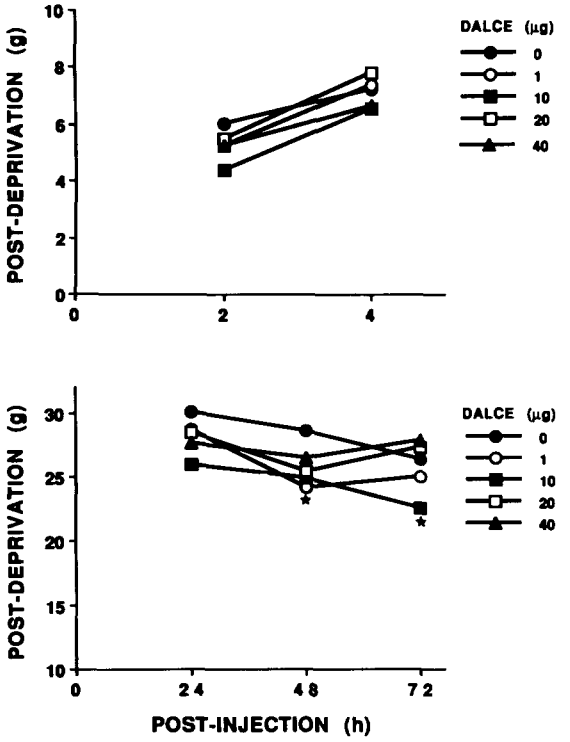

FIG. 2. Alterations in deprivation-induced intake (g) in rats treated 24 h earlier with either vehicle (n=18) or DALCE at doses of 1 (n=8), 10 (n=8), 20 (n=18) or 40 (n=9) µg after 2–4 (upper panel) and 24–72 h (lower panel) following reintroduction of food. The following are SEM ranges at 2 (0.5–1.3 g), 4 (0.5–1.4 g), 24 (1.2–4.8 g), 48 (0.8–1.9 g) and 72 (0.5–1.7 g) h.

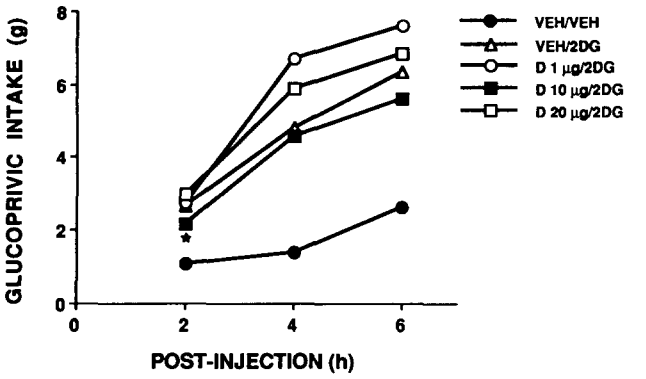

FIG. 3. Alterations in glucoprivation-induced intake (g) at 2, 4 and 6 h after administration of vehicle (n=15) or 2-deoxy-D-glucose (2DG, 650 mg/kg, IP) in rats treated 24 h earlier with either vehicle (n=15) or DALCE at doses of 1 (n=11), 10 (n=12) or 20 (n=15) µg. The following are SEM ranges at 2 (0.2–0.4 g), 4 (0.2–0.9 g) and 6 (0.4–0.8 g) h.

deprivation (Table 2). However, whereas the high 40-µg DALCE dose significantly retarded body-weight recovery 24 h after food reintroduction, body-weight recovery was significantly facilitated 48 h after food reintroduction following the 10-µg dose, and 48 and 72 h after food reintroduction following the 20-µg dose of DALCE (Table 2).

DALCE and Glucoprivic Feeding

Significant differences in glucoprivic feeding were observed among vehicle, 2DG and DALCE/2DG conditions at 2 h, $F(4,65)=4.61$, $p<0.0025$, 4 h ($F=14.07$, $p<0.0001$) and 6 h ($F=14.23$, $p<0.0001$), but not at 24 h ($F=0.83$) after systemic administration of vehicle or 2DG. Figure 3 indicates that 2DG significantly increased food intake following central pretreatment with vehicle at 2 (244%), 4 (343%) and 6 (241%) h but not at 24 h (8%, data not shown) after induction of glucoprivation. Long-term (24 h) pretreatment with DALCE (10 µg) significantly suppressed 2DG hyperphagia at 2 h by 30%. In contrast, long-term pretreatment with DALCE at a dose of 1 µg potentiated 2DG hyperphagia at 4 (55%) and 6 (35%) h.

DALCE and High-Fat Feeding

DALCE significantly altered intake of a high-fat diet after 1, $F(3,37)=5.85$, $p<0.002$, and 2 ($F=3.67$, $p<0.023$) h of exposure. Figure 4 indicates that long-term (24 h) pretreatment with DALCE (1 µg) significantly increased high-fat intake at 1 and 2 h after exposure to the diet.

Antagonist Effects Upon Short-Term DALCE Feeding

Significant differences in free feeding were observed among conditions at 2, $F(5,96)=15.83$, $p<0.0001$, 4 ($F=14.12$,

TABLE 1

TIME-RELATED ALTERATIONS IN BODY WEIGHT CHANGE (g, SEM) FOLLOWING VEHICLE AND DALCE RELATIVE TO PREINJECTION VALUES IN FREELY FEEDING RATS

DALCE Dose (µg)	Postinjection Interval (h) 24	48	72	96
0	−4.1(0.5)	+4.6(0.1)	+8.8(1.1)*	+11.7(1.0)*
1	−9.9(1.4)*	−3.3(3.1)	+0.6(2.7)	−2.6(1.9)
10	−8.2(0.1)*	−1.2(0.3)	+1.8(0.7)	+2.0(0.6)
20	−12.9(1.0)*	−2.2(0.4)	0.0(0.9)	+0.9(1.6)
40	−18.1(0.8)*	−6.5(0.9)	−3.9(0.3)	+0.5(1.1)

The asterisks denote significant changes in body weight relative to corresponding preinjection values, Dunnett comparisons ($p<0.05$).

TABLE 2

TIME-RELATED ALTERATIONS IN BODY WEIGHT CHANGE (g, SEM) FOLLOWING VEHICLE AND DALCE RELATIVE TO PREDEPRIVATION VALUES IN FOOD-DEPRIVED RATS

DALCE Dose (µg)	Post-deprivation	Postreintroduction (h) 24	48	72
0	−40.8(5.4)	−9.0(5.6)	−7.4(5.5)	−3.7(5.8)
1	−34.8(7.8)	−8.0(8.3)	−4.0(8.0)	−1.5(8.0)
10	−37.0(5.9)	−4.7(6.6)	−0.3(7.3)*	+1.4(6.5)
20	−38.9(6.9)	−6.3(6.4)	+1.3(6.1)*	+5.5(6.1)*
40	−43.0(11.3)	−20.0(16.4)*	−9.4(13.4)	−2.0(12.3)

The asterisks denote significant changes in body weight relative to corresponding vehicle injection values, Dunnett comparisons ($p<0.05$).

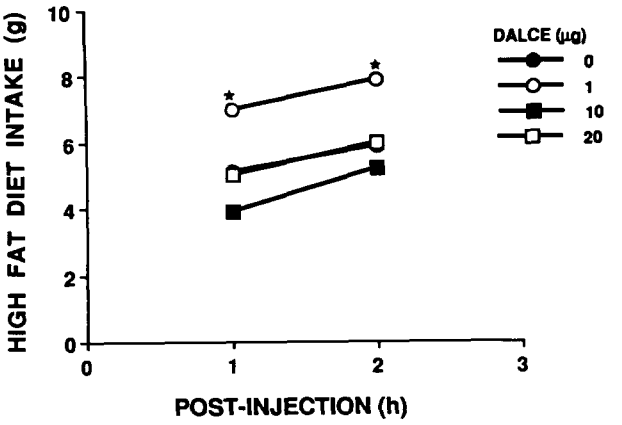

FIG. 4. Alterations in intake (g) of a high-fat diet over 2 h in rats treated 24 h earlier with either vehicle (n = 12) or DALCE at doses of 1 (n = 7), 10 (n = 11) or 20 (n = 11) μg. The following are SEM ranges at 1 (0.3–0.7 g) and 2 h (0.4–0.6 g).

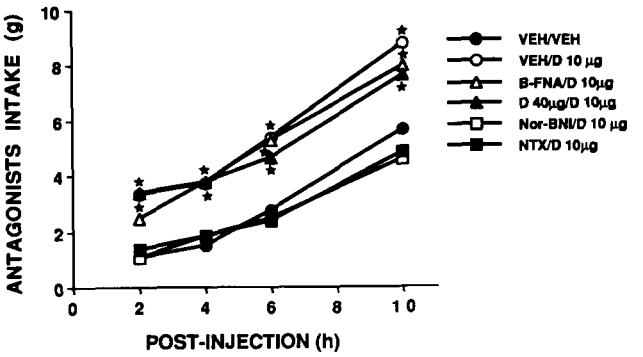

FIG. 5. Alterations in food intake (g) in rats treated with paired injections of either vehicle/vehicle (n = 26), vehicle/DALCE (10 μg; n = 26), nor-binaltorphamine (Nor-BNI, 20 μg)/DALCE (n = 12), naltrexone (NTX, 20 μg)/DALCE (n = 13), beta-funaltrexamine (B-FNA, 20 μg)/DALCE (n = 12) or DALCE (40 μg)/DALCE (n = 13). The respective intervals between injections for the first four and for the last two injection series were 1 h and 24 h, respectively. The following are SEM ranges at 2 (0.2–0.4 g), 4 (0.1–0.4 g), 6 (0.3–0.6 g) and 10 h (0.3–0.8 g).

$p<0.0001$), 6 ($F=14.49$, $p<0.0001$) and 10 h ($F=11.69$, $p<0.0001$) after the second vehicle or DALCE injection. Figure 5 indicates that the 10-μg dose of DALCE significantly increased food intake following central pretreatment with vehicle across the 10-h time course. The general opiate antagonist, naltrexone, administered 1 h prior to DALCE treatment, significantly inhibited DALCE hyperphagia at 2 (87%), 4 (84%), 6 (100%) and 10 h (100%) after the second injection. The kappa receptor antagonist, Nor-BNI, administered 1 h prior to DALCE treatment, significantly inhibited DALCE hyperphagia at 2 (100%), 4 (85%), 6 (100%) and 10 h (100%) after the second injection. In contrast, the mu receptor antagonist, B-FNA, administered 24 h prior to DALCE treatment, transiently and nonsignificantly altered DALCE hyperphagia at 2 (39% decrease), 4 (4% increase), 6 (4% decrease) and 10 h (26% decrease) after the second injection. Pretreatment with a 40-μg dose of DALCE 24 h earlier failed to significantly alter subsequent DALCE hyperphagia at 2 (2% increase), 4 (3% increase), 6 (28% decrease) and 10 h (38% decrease) after the second injection.

DISCUSSION

The selectivity of DALCE for delta receptors was initially confirmed in binding competition studies in which rat brain membranes were incubated with either unlabelled DALCE, DADL, DSLET or DPDPE in the presence of either ^{3}H-DPDPE (delta), ^{3}H-DAMGO (mu) or ^{3}H-bremazocine (in the presence of mu and delta blockers: kappa) (4). The IC_{50} for delta receptors of DALCE (4.1) was comparable to the other delta-selective ligands: DADL (3.4), DSLET (4.0) and DPDPE (5.5). Whereas the IC_{50} of DALCE (55) for mu receptors was 13-fold higher than for delta receptors, DALCE did exhibit greater affinity for mu receptors than DADL (151), DSLET (250) and DPDPE (6167) (4). Hence DALCE exhibits short-term agonist actions at delta and mu receptors and subsequent irreversible antagonist actions at delta receptors. These properties have been demonstrated in several models of analgesia (4, 6, 22). DALCE was used to further clarify the role of delta receptors in the mediation of the following forms of food intake: a) agonist effects upon free feeding, and antagonist effects upon b) free and deprivation feeding and c) glucoprivic and high-fat feeding.

Short-Term Agonist Actions of DALCE Upon Free Feeding

Following central administration of the 10-μg dose of DALCE, rats significantly and replicably increased food intake over 2–10 h. The magnitude (40–80%) of increased intake is comparable to that observed for other short-acting delta opioid agonists as DADL, DALA and DSLET (15, 16, 31, 48, 51). However, whereas these and other opioid agonists typically stimulate food intake for up to 4–6 h, DALCE increased intake over the 10-h test period in the light cycle. These data support the in vitro evidence of prolonged occupation of delta receptors by DALCE and suggest that DALCE may continue to act as an agonist at these receptors for some period of time. However, these stimulatory effects were not dose dependent, since lower (1 μg, ICV) and higher (20–40 μg, ICV) doses of DALCE failed to alter food intake. In contrast to monotonic dose-dependent actions of opioid agonists on pain thresholds [e.g., (56,57)], the failure to observe consistent dose-response curves for the stimulatory effects of opiate agonists upon food intake has been quite common, beginning with initial characterizations of opioid hyperphagia with morphine [e.g., (44)]. In addition to stimulation of feeding and analgesia, opioids exert other behavioral effects, including sedation, hypoactivity and immobility. Indeed, as reported previously (7), DALCE produced a transient, dose-dependent immobility. Therefore, the ability of higher DALCE doses to stimulate intake may have been compromised by subtle motor dysfunctions and/or slight sedation.

The opioid mediation of short-term DALCE hyperphagia was confirmed by the ability of naltrexone to block its expression. Interestingly, naltrexone blocked DALCE hyperphagia over its full 10-h time course, providing support for the view that DALCE stimulates food intake by its continued agonist actions on opioid receptors rather than some pharmacokinetic persistence of DALCE in the brain. Naltrexone is short-acting, losing its antagonist effects between 2 and 4 h after administration. If DALCE were stimulating feeding by short-term occupancy of opioid receptors but slow pharmacokinetic clearance in the brain, it would be expected that DALCE hyperphagia would return after the clearance of naltrexone. That naltrexone blocked the full duration of DALCE hyperphagia suggests that opioid-antagonist pretreatment prevented DALCE from exerting its 10-h stimulatory effect on intake by blocking initial attachment to the receptor site.

The particular receptor subtype that DALCE is stimulating to produce feeding is not consistent with the in vitro actions of

DALCE, which indicates high affinity for delta receptors, moderate affinity for mu receptors, and negligible affinity for kappa receptors (4). The kappa-selective antagonist, Nor-BNI, proved most effective in blocking the occurrence of DALCE hyperphagia over its full 10-h time course with the magnitude of antagonism ranging from 85–100%. Indeed, Nor-BNI displayed the same profile of antagonism as naltrexone. Prior administration of DALCE (24 h) has been shown to block the acute agonist actions of a subsequent dose of DALCE in antinociceptive assays (22). However, prior DALCE treatment failed to significantly alter the hyperphagic effects of subsequent DALCE administration; respective nonsignificant reductions of 28 and 38% occurred for intake 6 and 10 h after agonist treatment. Further, the mu-antagonist, B-FNA, failed to significantly alter DALCE hyperphagia, with nonsignificant reductions of 39 and 26% respectively noted 2 and 10 h after agonist treatment. This raises two possible explanations. First, DALCE stimulates food intake directly through weak kappa agonist effects and not through its delta or mu actions. This possibility would support the contention that kappa receptors are the integral subtype responsible for the opioid modulation of feeding (11, 15, 25, 26, 36–40). Second, DALCE stimulates food intake directly through delta *and* mu receptors which, in turn, require an intact kappa receptor-mediated system for its expression. In the latter model, the relative inability of either delta antagonism by DALCE alone or mu antagonism by B-FNA alone to block DALCE hyperphagia would be explained by the ability of DALCE to stimulate the remaining receptor subtype. This relies on the evidence [e.g., (15)] that different opioid receptor subtype agonists each stimulate food intake. Levine and co-workers (23) have recently suggested that the kappa receptor might form part of the final common pathway for opioid-induced feeding when they observed that the kappa-selective antagonist, Nor-BNI, equipotently reduced hyperphagia induced by the kappa-selective agonist, U50,488H, the mu-selective agonist, DAMGO, and the delta-selective agonist, DSLET. The ability of Nor-BNI to effectively eliminate DALCE hyperphagia supports this contention. Third, the inability of long-term pretreatment with DALCE to block the short-term hyperphagia induced by DALCE might also suggest that, whereas the expected (4) short-term delta agonist actions of DALCE occurred, the expected long-term delta antagonist actions of DALCE did not. Studies utilizing similar doses of DALCE indicated that long-term DALCE pretreatment selectively blocked analgesia induced by specific delta agonists (6, 7, 22). To confirm this possibility, future studies might examine whether: a) the agonist actions of short-term DALCE pretreatment are blocked by another selective delta antagonist such as naltrindole, and/or b) the delta-selective antagonist actions of long-term DALCE pretreatment block hyperphagia induced by highly selective delta agonists such as DSLET.

Long-Term Antagonist Actions of DALCE Upon Free and Deprivation Feeding

Whereas DALCE (1–40 μg) failed to reduce food intake after 24, 48, 72 or 96 h in freely feeding rats, it dose-dependently decreased body weight 24 h after administration. Moreover, the highest DALCE dose disrupted feeding efficiency 24 h after administration, as measured by the ratio of body weight to food intake. Whereas long-term DALCE pretreatment failed to alter short-term (2–4 h) deprivation-induced feeding and only sporadically decreased longer-term (24–72 h) intake following deprivation, only the highest DALCE dose significantly retarded body-weight recovery after 24 h in deprived rats. Indeed, lower DALCE doses stimulated body-weight recovery after 48 and 72 h in deprived rats. These data strongly suggest that the delta receptor might not be implicated in the opioid modulation of these forms of intake. As indicated previously (4), DALCE forms covalent attachments to the delta, but not the mu or kappa, receptors, thereby producing irreversible antagonism of this receptor subtype. In vivo confirmation of this selective antagonism was provided by the ability of DALCE pretreatment at 24, 48 or 72 h to block analgesia induced by DPDPE on the hot-plate and formalin tests without altering the analgesic actions of U50,488H or DAMGO (6, 7, 22). It should be noted that DALCE antagonism in these assays was noted at doses as low as 2 μg on the hot-plate test and 6.7 μg on the formalin test; the dose range utilized in the present study includes and goes beyond these effective doses. Jackson and Sewell (21) initially reported that the short-acting delta-selective antagonist, ICI 174864 (1–100 μg, ICV), produced dose- and time-dependent decreases in nocturnal free feeding, with the peak effects observed 1 and 2 h after injection. However, ICI 174864 produces motor dysfunction and paraplegia following intrathecal administration (28) and only reduced high-fat feeding following central administration at doses (10 μg) that were accompanied by motor dysfunction (18). The inability of DALCE to alter either free or deprivation-induced feeding, together with the possibility that ICI 174864 might be suppressing intake through motor dysfunction, suggest that the delta receptor is not integrally involved in these forms of feeding. Rather, the respective abilities of the kappa-selective antagonist, Nor-BNI (2,23), the mu-selective antagonist, B-FNA (3, 24, 52) and the mu_1-selective antagonist, naloxonazine (32,46), to reduce free feeding and deprivation-induced feeding as well as the ability of selective kappa receptor agonists to stimulate free feeding (11, 15, 25, 26, 36–40) indicate the importance of these subtypes in the opioid modulation of these forms of intake.

Long-Term Antagonist Actions of DALCE Upon Glucoprivic and High-Fat Feeding

DALCE pretreatment 24 h prior to 2DG glucoprivation or introduction of a high-fat diet failed to significantly alter the increased food intake induced by these manipulations. Indeed, long-term pretreatment with the lowest dose of DALCE stimulated high-fat feeding. Previous work has shown that the delta-selective antagonist, ICI 174864, failed to alter 2DG hyperphagia (20) and hyperphagia induced by exposure of a high-fat diet at doses that failed to produce motor dysfunction (18). These data suggest strongly that the delta receptor is not involved in the opioid modulation of these forms of intake. In contrast, the kappa receptor antagonist, Nor-BNI, significantly reduced intake following 2DG glucoprivation and introduction of a high-fat diet (2). Moreover, agonists at kappa receptors stimulate palatable intake (11, 12, 19, 27). Whereas the mu-selective antagonist, B-FNA, significantly reduced intake following 2DG glucoprivation and introduction of a high-fat diet (3,18), the mu_1-selective antagonist, naloxonazine, failed to exert effects (18,46). The ability of mu [$mu_1 + mu_2$: (41,55)], but not mu_1, receptor antagonists to modulate these effects implicates the mu_2 binding site. Therefore, kappa and mu_2 opioid receptors appear to account for the opioid modulation of glucoprivic and high-fat intake.

Conclusions

The inability of delta receptor antagonism with long-term pretreatment with DALCE to alter free feeding, deprivation-induced feeding, glucoprivic feeding, high-fat feeding, and DALCE hyperphagia argues against a central role for the delta receptor in these responses. The role of a given receptor in modulating a certain behavior can be evaluated through judicious use of selec-

tive agonists and/or antagonists; this approach has been used widely in the analysis of opioid effects in analgesia and food intake. As in analgesia [see reviews: (56,57)], agonists of different receptor subtypes each stimulate food intake under certain conditions, and one can conclude modulation by mu, kappa and delta receptors. As in analgesia, selective opioid antagonists can block agonist effects. In each of these cases, however, one is using pharmacological stimulation to produce a physiological change. Interestingly, opioid antagonists themselves exert minimal influence upon nociceptive thresholds (45, 56, 57). As indicated, certain selective mu and kappa opioid antagonists produce profound effects upon normal intake, interfering with the interior milieu which utilizes opioids to induce, in part, an ingestive response. These same antagonists also interfere with the opioid component necessary for ingestive adjustments following a deprivation challenge. Kappa and mu_2 receptors appear to play a role in the response to a glucoprivic challenge as well as the increased ingestion to a palatable high-fat diet. Since highly specific delta-selective agonists stimulate food intake, how might the delta receptor be involved in feeding, given the failure of delta antagonists to affect intake? Whereas mu, mu_1, mu_2 and kappa receptors might be in the direct synaptic chain that is essential for the expression of feeding behavior under different circumstances, the delta receptor might act as a modulator, fine-tuning those ingestive responses in which delta agonists stimulate intake. Distinctions between direct and modulatory receptor roles in feeding behavior may emerge as important determinants in opioid and, indeed, other pharmacological interventions of ingestive dysfunction.

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