Pharmacology Biochemistry and Behavior, Vol. 62, No. 3, pp. 493–498, 1999

0091-3057/99 $–see front matter

PII S0091-3057(98)00213-5

DA_1 Receptor Activity Opposes Anorectic Responses to Amino Acid-Imbalanced Diets

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Received 15 August 1997; Revised 15 June 1998; Accepted 15 August 1998

AJA, S. M., P. CHAN, J. A. BARRETT AND D. W. GIETZEN. *DA_1 receptor activation opposes anorectic responses to amino acid-imbalanced diets.* PHARMACOL BIOCHEM BEHAV **62**(3) 493–498, 1999.—The serotonin$_3$ (5-HT_3) receptor plays an important role in the aminoprivic feeding model. Other neurochemical systems, including cholecystokinin (CCK) and dopamine (DA), are known to affect food intake. We pretreated rats systemically with tropisetron, a 5-HT_3 receptor antagonist, alone and combined with antagonists of DA_1 and DA_2 receptors, and measured intake of an amino acid-imbalanced diet (IMB). As expected, tropisetron significantly increased intake of IMB. SCH-23390, a DA_1 antagonist, increased IMB anorexia. When combined with tropisetron, DA_2 antagonism with eticlopride reduced short-term intake of both the basal diet (BAS) and IMB. In the IMB model, specificity of 5-HT_3–DA_2 interactions, and of 5-HT_3–CCK_A interactions from previous studies, prompted investigation of CCK_A–DA_2 interactions; there appeared to be none. SKF-38393, a DA_1 agonist, combined with the CCK_A receptor antagonist, devazepide, increased BAS and tended to increase IMB intake. Thus, CCK_A–DA_1 interactions were not specific for IMB. These data suggest that DA_1 receptor activity opposes IMB anorexia, possibly via an interaction with the 5-HT_3 receptor. © 1999 Elsevier Science Inc.

Amino acid deficiency Nutrition Food intake Tropisetron Devazepide SCH-23390
SKF-38393 Eticlopride Serotonin$_3$ receptor CCK_A receptor DA_1 receptor DA_2 receptor

OMNIVORES are able to select a complete diet containing all of the amino acids required for protein synthesis from incomplete sources. This selection depends on the ability to recognize the metabolic consequences of ingesting a diet that contains an amino acid deficiency or imbalance. This homeostatic system has been well studied behaviorally (17), but the neural mechanisms that underlie the ability to recognize amino acid deficiency are not fully understood. Rats prefed low-protein basal diets consistently show recognition and rejection of an amino acid-imbalanced diet (IMB) by reducing their food intake (17,22,23). Onset of the anorectic response is rapid, and can be seen within 1/2 h, reaching significance within 1–2 h, depending on the degree of amino acid disproportionality and the prefeeding regimen (10,23). Serotonin (5-HT) appears to be involved in the reduced intake of IMB (12), an effect selective for the 5-HT_3 receptor (16,21,25), because the 5-HT_3 antagonist, [1H]-indole-3-carbonic acid-tropine-ester hydrochloride, tropisetron (TROP, formerly ICS 205-930),, MDL 72222, and ondansetron all ameliorate the anorectic response (21). Our laboratory has shown increases in metabolite/serotonin (5-HIAA/5-HT) ratios in several brain areas after introduction of IMB (11), and ondansetron, a selective 5-HT_3 antagonist, injected into the anterior piriform cortex, increases IMB intake (13,25), suggesting a central site for 5-HT in the response to IMB. However, central injections of into the cisterna magna and lateral ventricle do not affect the feeding depression associated with IMB (20). Systemic injections of TROP or its quaternized form attenuate the anorectic response equally (20). Thus, peripheral 5-HT_3 receptors are likely to be involved in the depressed consumption of amino acid-imbalanced diets, as well. Still, the finding that pretreatment with TROP yields IMB intakes that are only 80–85% of baseline basal diet (BAS) intake (16) prompted us to ask whether the 5-HT_3 receptor acts alone in the responses to IMB, or whether other systems may be involved. Since the initial proposal for the involvement of serotonin in feeding control (5), considerable research has demonstrated potential interactions between serotoninergic activity and that of other systems. Dopamine (DA) is implicated in reinforcement of feeding responses (19), and many studies demonstrate that

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5-HT_3 receptor activation enhances DA release from limbic structures (4,6). Therefore, the 5-HT_3 system may also interact with DA systems in the control of food intake.

The present studies were designed to evaluate potential interactions between TROP and activity at DA_1 and DA_2 receptors in the IMB feeding model, using peripheral injections of selective antagonists and agonists of the receptors in question. Preliminary data from these trials and from a previous study [(1), the companion paper] prompted investigation of CCK_A-DA interactions as well. We hypothesized that improving the antianorectic potential of the 5-HT_3 antagonist in this nutritional model with agents acting selectively at other receptors known to be involved in feeding could suggest which systems interact in the feeding responses to IMB. The systems used in the present study were serotonin, at the 5-HT_3 receptor, because of our previous work with this receptor (16,20), CCK at the CCK_A receptor, based on the results of the companion paper (1) and DA, because it has important effects on feeding, and because our previous work (16) showed an intermediate response to a nonselective dopamine antagonist, leading us to suspect that the DA_1 and DA_2 receptors might have reciprocal effects in our system.

METHOD

Animals

Sprague–Dawley male rats (Simonsen Labs, Gilroy, CA) were naive to diet and drug treatments. We used young, growing rats in our studies because they are exquisitely sensitive to IMB. Animals were housed individually in hanging wire cages in a controlled environment maintained at 22 ± 2°C, on a 12 L:12 D cycle, with onset of the dark phase at noon. Animal protocols were approved by the University's Animal Use and Care Committee. Purified L-amino acid diets (Table 1) and water were available ad lib. The rats were allowed at least 10 days to adjust to a powdered low-protein isoleucine (ile) basal (BAS) diet (Table 1), housing conditions, and the food intake protocol. After 3 days of baseline food intake measurements on the BAS diet, the animals were weighed and randomly assigned to experimental groups having equal mean body weights. On the first experimental day (ED1), food cups were removed and replaced with fresh cups of either ile-BAS or ile-IMB diet (Table 1). The rats were given IP injections of the drugs or equal volumes of the appropriate vehicles 10–45 min before onset of the dark cycle, at which time preweighed cups containing the test diets were placed in the cages. Food intake, in g/interval, represents the difference between food cup weights before and after the interval, corrected for spillage. Food intake measurements were taken at 3-h intervals during the dark cycle, with a subsequent 12-h measurement of feeding during the light cycle. Daily food intake measurements were continued for 3 days after the injections. Specific food intake protocols are described for each experiment.

TABLE 1
COMPOSITION OF DIETS USED IN EXPERIMENTS
% OF DIET BY WEIGHT

Ingredients	BAS	IMB
Dispensable amino acid mixture	7.53	7.53
Indispensable amino acid mixture	3.77	3.77
Imbalanced amino acid mixture		9.10
Vitamin mixture	1.00	1.00
Salt mixture	5.00	5.00
Corn oil	5.00	5.00
Sucrose	25.87	22.83
Cornstarch	51.73	45.67
Choline chloride	0.10	0.10
Total	100.00	100.00

Isoleucine was the growth limiting amino acid in both diets. BAS, ile-basal diet; IMB, ile-imbalanced diet. All ingredients have been described previously in detail (16).

Diets

Purified diets, with L-amino acids as the sole protein source (Ajinomoto, USA, Inc., Teaneck, NJ) and ile as the growth-limiting amino acid, were used in the experiments. The amino acid-imbalanced diet (IMB) was prepared by adding indispensable amino acids, except Ile, to the low protein BAS diet (Table 1). These diets have been described in detail previously (16).

Drugs

TROP was a gift from Sandoz Research Institute (East Hannover, NJ). 3S-(−)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-1H-indole-2-carboxamide (Devazepide, DEV, formerly L-364,718) was a gift of Merck, Sharp and Dohme Research Laboratories (West Point, PA). R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benazepine hydrochloride (SCH-23390, SCH), R-(+)-1-phenyl-7,8-dihydroxy-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SKF-38393, SKF), and S-(−)-3-chloro-5-ethyl-N-[(1-ethyl-2-pyrrolidinyl)methyl]-6-hydroxy-2-methoxy-benamide hydrochloride (Eticlopride, ETIC) were purchased from Research Biochemicals International (Natick, MA). The doses were selected from previous dose–response studies for TROP (16,21), and trials using SCH, SKF, and ETIC in animals fed the BAS diet (Table 2). Therefore, where BAS intake was decreased by the drug, we selected an intermediate dose. TROP was prepared in 0.9% NaCl (Sal) and administered intraperitoneally at a dose of 9 mg/kg body weight. DEV was dissolved in 4% ethanol (EtOH) and given at 0.1 mg/kg. SCH and ETIC were dissolved in Sal and administered at 0.1 mg/kg. SKF was dissolved in Sal and given at 5 mg/kg. Each drug or vehicle was injected IP in a volume of 1 ml/kg.

Data Analysis

Food intake data were subjected to analysis of variance (ANOVA), using a general linear model (GLM) with type III sums of squares (version 6.04, SAS, Cary, NC). Diet and drug were the independent variables; interactions among diets and drugs were also examined. Food intake, the dependent variable, was expressed as g/interval/rat. Least-significant difference tests were performed to compare group means after a significant overall ANOVA (Fischer's protected LS mean). Significance was assumed at $p \leq 0.05$.

Experiment 1a: 5-HT_3 and DA_1 Receptor Interactions

Tropisetron and SCH-23390. TROP and SCH, a DA_1 receptor blocker, were employed to investigate interactions between 5-HT_3 and DA_1 systems in the IMB-feeding model. Rats weighed 180–200 g at the beginning of Experiments 1a and 1b. A 4 × 2 factorial design was used, with eight rats per group. The two diets were BAS and IMB. The four drug conditions were Sal+Sal, Sal+TROP, SCH+Sal, and SCH+TROP. BAS diet intake was measured at 3-, 6-, 9-, 12-, and 24-h inter-

TABLE 2
DOSE–RESPONSE DATA: CUMULATIVE FOOD INTAKES ON AN ILE-BAS DIET

ED1 Intervals (h)	Doses			
	Control	Low	Medium*	High
		SCH-23390		
0–3	3.44 ± 0.56[b]	2.75 ± 0.47[b]	0.96 ± 0.17[a]	0.012 ± 0.012[a]
0–6	7.11 ± 0.62[c]	6.03 ± 0.40[c]	4.28 ± 0.32[b]	2.31 ± 0.26[a]
0–9	9.93 ± 0.78[c]	9.75 ± 0.51[c]	7.45 ± 0.28[b]	5.44 ± 0.41[a]
0–12	12.87 ± 0.91[b]	12.77 ± 0.33[b]	10.66 ± 0.46[a]	10.03 ± 0.88[a]
0–24	16.66 ± 0.50[a]	17.38 ± 0.69[a]	16.42 ± 0.44[a]	17.16 ± 0.77[a]
		Eticlopride		
0–3	4.69 ± 0.49[b]	4.87 ± 0.59[b]	3.70 ± 0.60[b]	0.75 ± 0.17[a]
0–6	8.56 ± 0.47[c]	9.31 ± 0.77[c]	6.65 ± 0.65[b]	0.75 ± 0.17[a]
0–9	11.30 ± 0.43[c]	12.23 ± 0.95[c]	8.53 ± 1.00[b]	0.65 ± 0.20[a]
0–12	14.69 ± 0.72[bc]	15.71 ± 1.32[c]	11.97 ± 1.07[b]	0.67 ± 0.20[a]
0–24	17.12 ± 0.65[b]	18.37 ± 1.41[b]	18.96 ± 1.26[b]	1.17 ± 0.13[a]
		SKF-38393		
0-3	4.56 ± 0.17[b]	4.79 ± 0.65[b]	2.58 ± 0.25[a]	2.14 ± 0.13[a]
0–6	8.86 ± 0.30[b]	9.18 ± 0.44[b]	6.59 ± 0.18[a]	7.25 ± 0.57[a]
0–9	12.43 ± 0.49[b]	12.45 ± 0.37[b]	11.35 ± 0.45[ab]	10.31 ± 0.37[a]
0–12	15.28 ± 0.61[a]	15.74 ± 0.82[a]	14.32 ± 0.46[a]	14.13 ± 0.68[a]

Values are means ± SE; $n = 6$ animals/group. Data are cumulative food intakes expressed in grams of diet eaten. Drugs and doses: SCH-23390 and eticlopride (mg/kg body weight): low = 0.01, medium = 0.1, high = 1.0; SKF-38393 (mg/kg): low = 1.0, medium = 5, high = 10; all drugs; control = 0. Drugs were dissolved in 0.9% NaC1 (1 m1/kg). Values with differing superscript letters within an experiment and within a time interval are significantly different ($p \leq 0.05$).

*Medium dose selected for present studies.

vals for 3 days. On ED1, rats were injected with drugs and vehicles prior to access to the test diets. Food intake was measured at 3, 6, 9, 12 and 24 h on ED1, and at 24 h only on the second and third days of the experiment (ED2, ED3).

Experiment 1b: 5-HT_3 and DA_2 Receptor Interactions

Tropisetron and eticlopride. In consideration of the well-known emetic effects of the DA_2 agonist, apomorphine (14), we used ETIC, an antagonist at DA_2 receptors, with TROP to evaluate interactions between the DA_2 and 5-HT_3 systems in the imbalanced feeding model. The design of Experiment 1a was repeated, substituting the DA_2 receptor antagonist for the DA_1 blocker. The food intake and drug administration protocols were the same as those of Experiment 1a.

Experiment 2a: CCK_A and DA_2 Receptor Interactions

Devazepide and Eticlopride. Results from previous studies (1) and from Experiments 1a and 1b indicated that activity at CCK_A and both DA receptors might interact with the 5-HT_3 system in the feeding model. In Experiment 2a, potential interactions between the CCK_A and DA_2 systems were examined. Rats weighed 200–220 g at the beginning of the experiment. Six rats were assigned to each group in the same 4×2 factorial design as in the experiments above. EtOH+Sal, DEV+Sal, EtOH+ETIC, and DEV+ETIC were the four drug conditions. Again, the diets were BAS and IMB. BAS intake was measured for 3 days at 3, 6, 9, 12, and 24 h. Drugs and vehicles were injected on ED1 as described previously. Test diet intake was measured for 3 days, at 3, 6, 9, 12, and 24 h on ED1, and at 24 h only on ED2 and ED3.

Experiment 2b: CCK_A and DA_1 Receptor Interactions

Devazepide and SKF-38393. The results of Experiment 1a indicated that 5-HT_3 and DA_1 may interact in the control of IMB intake, and earlier experiments (1) suggested that cooperation between 5-HT_3 and CCK_A receptors mediate IMB anorexia. Thus, it was important to determine if the CCK_A and DA_1 systems might interact in the control of intake of IMB. SCH, the DA_1 antagonist used in Experiment 1a, tended to reduce feeding of IMB, and thus might have counteracted the orexigenic effect of the CCK_A antagonist, resulting in a masking of the effects of these two systems. Therefore, the DA_1 agonist, SKF, was given in conjunction with the CCK_A antagonist, DEV, in this experiment. Rats weighed 165–195 g at the beginning of the experiment. Six rats were assigned to each group in a 4×2 factorial design. The four drug conditions were EtOH+Sal, DEV+Sal, EtOH+SKF, and DEV+SKF. The diets were BAS and IMB. Baseline BAS intake was measured for 3 days at 3, 6, 9, 12, and 24 h each day. Drugs and vehicles were injected on ED1 as described previously. Test diet intake was measured for 3 days at 3-, 6-, 9-, 12-, and 24-h time points.

RESULTS

Confirming previous findings (17), double vehicle-treated rats consistently responded to IMB with reduced intake, relative to their BAS intake, in all experiments (Figs. 1–3). The anorectic response was significant by 6 h in most cases. Pretreatment with TROP significantly attenuated the IMB anorexia by 6 or 12 h when compared with the IMB: vehicle-treated controls (Figs. 1 and 2). We have consistently seen an

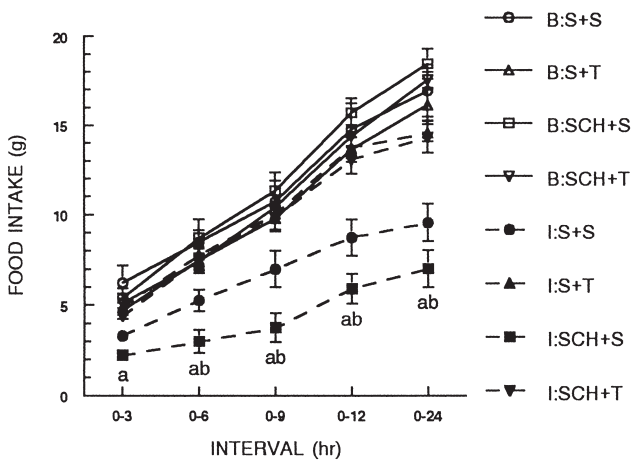

FIG. 1. Experiment 1a: food intakes on experimental day 1. Values are means ± standard errors; $n = 6$ animals/group. Data are expressed in grams of diet eaten. Diets: B, ile-basal diet; I, ile-imbalanced diet. Drugs and vehicles: S, 0.9% NaCl vehicle (1 ml/kg); the 5-HT_3 antagonist, T, tropisetron (9 mg·ml^{-1}·kg^{-1}) in S; the DA_1 receptor antagonist, SCH, SCH-23390 (0.1 mg·ml^{-1}·kg^{-1}) in S. Lower case letters indicate significant differences from vehicle control in the IMB groups: (a) SCH-233909 less than control or SCH × diet interaction (see text), (b) TROP greater than control ($p < 0.05$).

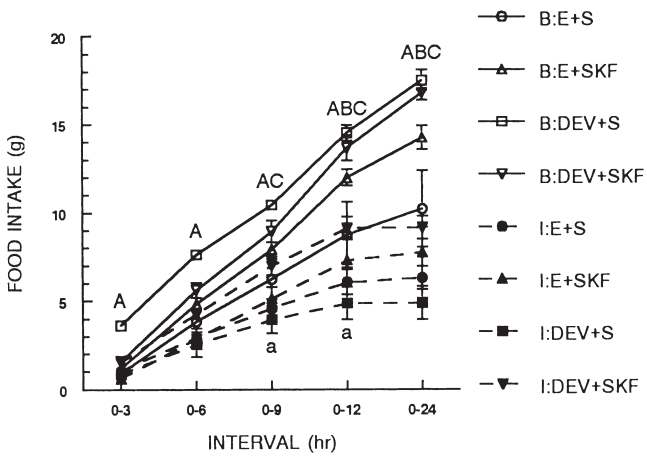

FIG. 3. Experiment 2b: food intakes on experimental day 1. Values are means ± SE; $n = 6$ animals/group. Data are expressed in grams of diet eaten. Diets: B, ile-basal diet; I, ile-imbalanced diet. Drugs and vehicles: E, 4% ethanol vehicle (1 ml/kg); S, 0.9% NaCl vehicle (1 ml/kg); the CCK_A antagonist, DEV, devazepide (0.01 mg·ml^{-1}·kg^{-1}) in S; the DA_1 receptor agonist, SKF, SKF-38393 (5 mg·ml^{-1}·kg^{-1}) in S. Letters indicate significant differences from the respective vehicle control as follows: capital letters, differences within BAS diet groups: (A) DEV greater than control, (B) SKF greater than control, (C) DEV + SKF greater than control. Lower case letters indicate significant differences from vehicle control in the IMB groups: (a) DEV + SKF greater than control ($p < 0.05$).

increase in IMB intake within 6–9 h after introduction of the IMB, in TROP-treated animals (16,20).

Experiment 1a: 5-HT_3 and DA_1 Receptors

During the first 3 h of ED1 there were significant differences in food intake, as shown in Fig. 1, $F(7, 57) = 6.23$, $p = 0.0001$. Intake of BAS by the TROP group was less than that of the Sal+Sal group, but only at this time ($p = 0.030$). SCH administered alone had no effect on BAS intake, but significantly decreased IMB intake, beginning at this time point, and continuing throughout the rest of ED1 ($p \leq 0.03$).

By 24 h, $F(7, 57) = 18.33$, $p = 0.0001$, the SCH effect on IMB intake was no longer significant ($p = 0.06$), although there was a significant SCH × diet interaction ($p = 0.035$). TROP alone increased IMB intake to BAS control levels through 12 h, ($p = 0.25$), and the SCH+TROP group did not differ from TROP alone (Fig. 1).

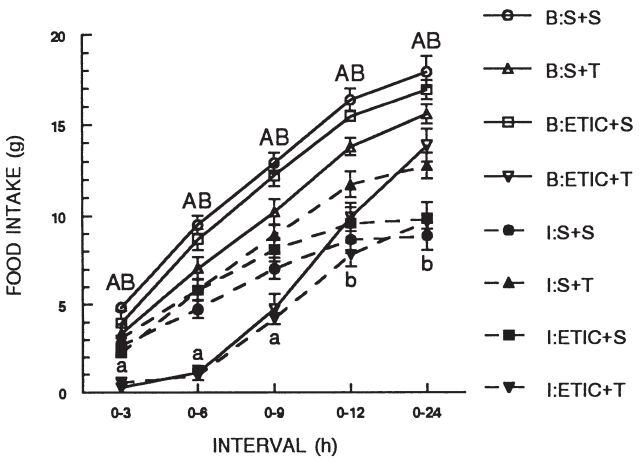

FIG. 2. Experiment 1b: food intakes on experimental day 1. Conditions are the same as in Fig. 1 except for the DA antagonist, which was eticlopride, selective for the DA_2 receptor, abbreviated ETIC, in place of SCH-23390. Letters indicate significant differences from the respective vehicle control as follows: capital letters, differences within BAS diet groups: (A): ETIC + TROP less than control; (B) TROP less than control. Lower case letters indicate significant difference from vehicle control in the IMB groups: (a) ETIC + TROP less than control, (b)TROP greater than control ($p < 0.05$).

Experiment 1b: 5-HT_3 and DA_2 Receptors

At 3 h, an anorectic response to the combination of ETIC and TROP, in both BAS and IMB groups, was remarkable [overall, $F(7, 54) = 13.45$, $p = 0.0001$], and again, TROP alone decreased BAS intake from the BAS:vehicle group ($p = 0.023$) (Fig. 2). The hypophagia after injections of ETIC+TROP lasted for 9 h in both BAS and IMB groups, and throughout ED1 for the BAS group, compared with double vehicle controls. TROP increased IMB intake significantly over double-vehicle control ($p = 0.001$) in the 0–12 h and 0–24 h measurements. ETIC alone did not alter either BAS or IMB intakes from double-vehicle controls at any time. There was a significant interaction between TROP and ETIC ($p = 0.034$–0.0009) for each time interval, except the 0–9-h measurement, for which a trend was noted ($p = 0.06$).

Experiment 2a: CCK_A and DA_2 Receptors

Because of the significant ETIC × TROP interaction seen in Experiment 1b, and after our observations that CCK_A and 5-HT_3 systems might interact in our model (1), potential interactions between the CCK_A and DA_2 systems were investigated (data not shown). After 3 h on ED1 [overall, $F(7, 40) = 10.44$, $p = 0.0001$], DEV had no effect on IMB anorexia, nor did it change intake of the BAS diet, whether given with ETIC or

not. Throughout the remainder of ED1, it was clear that neither DEV nor ETIC, given alone or in combination, had any effect on IMB anorexia. At 24 h, BAS intake was higher after ETIC injection, with or without DEV (ETIC effect, $p \leq 0.05$), when compared with the EtOH+Sal group ($p \leq 0.03$). There was no significant effect of DEV alone on either diet.

Experiment 2b: CCK_A and DA_1 Receptors

DEV increased BAS intake over the vehicle control group; the effect lasted all day ($p = 0.002$), as can be seen in Fig. 3. For the first 3 h, IMB and the remaining BAS groups did not differ. In the 3–24-h period BAS diet results were more typical, and the normal response to IMB was clearly evident in the double-vehicle controls ($p = 0.0058$). During the 3–24-h interval on ED1, DEV alone had no significant effect on IMB. SKF alone significantly increased BAS intake ($p = 0.011$), but had no effect on IMB intake. The use of both drugs together increased BAS intake significantly over control ($p = 0.0001$), but not to a level that was significantly different from the effects of each drug alone. DEV and SKF together also increased IMB intake compared with the double-vehicle control group during the 0–9-h and 0–12-h time periods ($p \leq 0.05$).

Summary of Experimental Days 1–3 Over All Trials

Data over the 3 days subsequent to drug or vehicle treatment showed that the food intakes of groups eating BAS remained near 100% of pretreatment control throughout all of the trials. In Experiment 2b, all groups increased their intake of IMB on ED3 (mean = 14.48 g) in the adaptation that can be seen with these diets (10,17), but they still ate less IMB (all $p < 0.001$) than they had of BAS during baseline BAS intake measurement (mean baseline BAS intake of IMB groups = 19.58 g).

DISCUSSION

The 5-HT_3 receptor appears to be essential for mediating the normal reduction in intake of essential amino acid-imbalanced diets (16). However, other neurochemical systems, notably those involving CCK and DA, also affect food intake. Our aim was to evaluate potential involvement of DA systems, and interactions of DA with 5-HT or CCK, in the aminoprivic feeding model. Our results indicate that the DA_1 system opposes the reduction in IMB intake.

DA and 5-HT_3 Receptors in Reward/Aversion

A current hypothesis regarding DA and 5-HT in feeding is the feeding-reward/aversion model (19), in which DA is thought to reinforce approach behavior to positive reinforcing stimuli. However, the potencies of DA_1 and DA_2 antagonists varied inversely with the reward value of sham-fed liquid nutrients (27). In an earlier study from our group (16), a nonselective DA antagonist produced an intermediate antianorectic effect on IMB intake. We hypothesized that this could have been due to reciprocal actions at the two receptor subtypes. The DA_2 receptor agonist, bromocriptine, reduced intake of both BAS and IMB diets at all doses tried in a previous study in our laboratory (unpublished results), decreasing food intake generally. Moreover, stimulation of the DA_2 system with apomorphine is associated with nausea and emesis (14).

Although DA is thought to be a positive reinforcer of feeding, 5-HT is implicated in negative reinforcements in conditioned cessation of feeding, or in the inhibition of positive reinforcements. The overall concept is supported by a body of evidence including, but not limited to, implications of nucleus accumbens DA involvement in reinforcement of feeding reward (18), and amygdala and hypothalamic 5-HT as integral to conditioned taste aversions (15,28). The dorsal raphe 5-HT system innervates many limbic structures that receive dopaminergic input from the ventral tegmental area (VTA) (the mesolimbic dopaminergic system), including the amygdala, nucleus accumbens, and piriform cortex (3,8,9,24), providing an anatomical rationale for this hypothesis.

Although SCH-23390, a DA_1 receptor antagonist, decreased intake of IMB without altering BAS diet intake (Fig. 1), SKF-38393, a DA_1 receptor agonist, did not affect IMB diet intake, but increased intake of BAS (Fig. 3) . These data seem consistent with the feeding-reward/aversion hypothesis (19) if IMB can be considered a negatively reinforcing stimulus and if BAS, by comparison, is fairly rewarding. We have not tested the reward value of BAS formally.

DA_1 and 5-HT_3 Receptors

SCH alone decreased intake of IMB in Experiment 1a, indicating that stimulation of the DA_1 receptor increases IMB intake (Fig. 1). SKF, a DA_1 receptor agonist, did not increase intake of IMB in Experiment 2b (Fig. 3). This may be due to a "ceiling effect" of endogenous DA at DA_1 receptors in rats eating IMB, so that further stimulation of DA_1 receptors would not result in an increase in IMB intake. Prevention of a more severe anorectic response to IMB by DA_1 activity could be important in our model.

SCH+TROP produced an IMB intake not significantly different from IMB:Sal+TROP at any time point (Fig. 1), so SCH did not alter the TROP effect. However, this may not be definitive proof of the lack of a DA_1–5-HT_3 interaction. Recent evidence indicates that activation of 5-HT_3 receptors can enhance mesolimbic DA activity: similar increases in DA release were elicited from the rat striatal slice with both 5-HT and 2-methyl-5-HT, a selective 5-HT_3 agonist, and such DA release was blocked with TROP at doses selective for 5-HT_3 receptors (4). Similar observations have been made in the nucleus accumbens in vivo (6). TROP may not fully remediate IMB feeding if it decreases DA transmission at DA_1 receptors via antagonism of 5-HT_3 receptors. Such an explanation could account for the lack of difference between IMB:SCH+TROP and IMB:Sal+TROP, because the DA_1 receptors would already be blocked by SCH in the IMB:SCH+TROP group.

DA_2 and 5-HT_3 Receptors

ETIC alone did not alter either BAS or IMB intake. The combination of ETIC and TROP decreased BAS and IMB intakes dramatically during the first 6 h of Experiment 1b (Fig. 2). Thus, DA_2 and 5-HT_3 systems may cooperate in a short-term increase or maintenance of feeding in general. More likely, the combination of ETIC and TROP may have made the animals temporarily ill and unable to eat. Supporting this, the 6–24 h data indicated a compensation for the early anorexia in the ETIC+TROP group. We do, however, conclude that the DA_2 receptor is not involved in the responses to IMB in any selective manner.

CCK_A and DA Receptors

Because we observed a decrease in intake of IMB with SCH, and because we saw interactions between CCK_A and 5-HT_3 [(1), companion paper], we investigated potential interactions between CCK_A and DA_1. CCK and DA from the VTA are proposed to interact closely at both presynaptic ter-

minals and postsynaptic cells in the nucleus accumbens (26), and CCK may play a role in regulating mesolimbic DA transmission (7). Neither DEV nor SKF significantly altered IMB intake from double vehicle control levels when given alone (Fig. 3). Combined treatment with the CCK_A antagonist and the DA_1 agonist increased both BAS and IMB intake. Thus, any potential interactions between the CCK_A and DA_1 systems were not specific to the IMB diet. In addition, the results with DEV and ETIC (Experiment 2a) clearly show that the CCK_A and DA_2 systems do not interact in the aminoprivic feeding model.

CONCLUSIONS

We show that activity at DA_1 receptors opposes IMB anorexia. 5-HT_3 receptor antagonists can depress mesolimbic DA release, and we offer this as a partial explanation for the incomplete increase of IMB intake with TROP in the present data, as well as in our previous work. 5-HT_3 and DA_2 systems may cooperate to support generalized, short-term increases in feeding, or the antagonists used in this study may have caused temporary illness. If there is an interaction between 5-HT_3 and DA_2 receptors that is relevant physiologically to the control of food intake, it does not appear selective for IMB feeding. Despite potential 5-HT_3–CCK_A [(1), companion paper) and DA_1 effects, we found no CCK_A–DA_1 interactions specific to IMB feeding. We also found no interactions between CCK_A and DA_2 receptors in our aminoprivic feeding model.

ACKNOWLEDGEMENTS

This research was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK42274 and NS33347 to D. W. Gietzen, DK-09271 to S. M. Aja, and DK-35747 to the Clinical Nutrition Research Unit of the University of California–Davis, and by a generous gift from Sandoz Research Institute. We also thank Jennifer Theisen, Ashwini Telang, and Anthony Mistry, a participant in the NSF Young Scholars Program, for their assistance with data collection and animal care. Preliminary data from experiment series 1 have been published in abstract form (2).

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