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Dopamine-Beta-Hydroxylase Inhibitors, Feeding and Locomotor Activity: Reinstatement of Feeding Following Central Norepinephrine[1]

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EVANS, K. R., R. J. BENINGER AND R. EIKELBOOM. *Dopamine-beta-hydroxylase inhibitors, feeding and locomotor activity: Reinstatement of feeding following central norepinephrine.* PHARMACOL BIOCHEM BEHAV **22**(4) 535–540, 1985.—The effects of two dopamine-β-hydroxylase (DBH) inhibitors, FLA-63 and fusaric acid (FA) on feeding behaviour and locomotor activity were examined. In Experiment 1 activity was measured over 7 hr in 48 rats treated with FLA-63 (0, 1.25, 2.50, 5.00 mg/kg) or FA (0, 20.0, 40.0, 80.0 mg/kg). While FA produced no significant effect on activity, FLA-63 produced an increase at the highest doses. In Experiment 2 the same doses of the two DBH inhibitors were administered to 48 rats and food intake over 7 hr was measured; both FA and FLA-63 produced decreases in food intake. In a third experiment, rats were stereotaxically implanted with microinjection guide cannulae extending to the ventromedial hypothalamus and, following peripheral treatment with either 5.0 mg/kg FLA-63, 40 mg/kg FA, or their respective vehicles were injected centrally with morphine (5.3 nmoles in 0.5 μl), norepinephrine (NE; 60 nmoles in 0.5 μl for the FLA-63 pre-treated group and 30 nmoles in 0.5 μl for the FA pre-treated group) or saline. Central NE was found to reinstate feeding only in the hr following injection in both groups, while morphine reinstated feeding only in the FA group and only in the third hr following injection. Results support the involvement of hypothalamic NE in feeding.

Dopamine-beta-hydroxylase inhibitors FLA-63 Fusaric acid Norepinephrine Morphine
Ventromedial hypothalamus Central injection Feeding Locomotor activity

NOREPINEPHRINE (NE) injected into the ventromedial hypothalamus (VMH) elicits a vigorous feeding response [5, 11, 24] suggesting a role in feeding. Administration into other areas of the hypothalamus also elicits feeding [13]. NE-induced feeding can be blocked by the α-adrenergic receptor blocker, phentolamine, but not the β-adrenergic blocker, propanolol [5, 12, 21]. However, phentolamine increases feeding when injected centrally or peripherally [15,22].

Numerous approaches have been taken to study the mechanisms of NE involvement in feeding (see [13] for a review). For example, intracerebral injections of 6-hydroxydopamine cause an initial release of NE and produce increased food intake [8]. However, other neurotransmitters which may have a role in feeding (e.g. dopamine) [13] are also affected, and damage is permanent and widespread [3].

The present work involved the use of dopamine-β-hydroxylase (DBH) inhibitors. DBH is the enzyme necessary for the conversion of dopamine (DA) to NE. Fusaric acid (FA) and FLA-63 (bis[4-methyl-1-homopiperazinyl-thiocarbonyl] disulphide), a compound structurally related to disulphiram, have been shown to inhibit DBH [23,28] and decrease levels of NE, presumably through inhibition of NE synthesis [2, 6, 23, 28]. The inhibition produced by FA, unlike disulphiram and related compounds, is non-competitive and does not seem to involve the chelation of copper [18].

The effects of DBH inhibitors on feeding are unclear. Friedman, Starr and Gershon [10] reported that intrahypothalamic injection of FLA-63 (40 μg) in 22 hr food deprived animals increased food intake. Conversely, Rossi, Zolovick, Davies and Panksepp [20] found that peripheral FLA-63 (10 mg/kg) had little effect in 22 hr food deprived animals but reduced feeding in non-deprived animals. Feeding was also reduced in deprived animals treated with FLA-63 (10 mg/kg) in combination with a monoamine vesicular reuptake blocker, an effect reversable by intrahypothalamic injections of NE (40 μmoles in 2 μl). However, the dose of NE was larger than that typically used in central administration experiments, e.g., 30 nmoles [24], and the volume was larger than optimal [17]. Thus, non-specific or remote effects could have produced the increased feeding. Therefore the effects on feeding of smaller doses and volumes of NE in animals treated with FLA-63 should be examined. Further, these findings should be replicated

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with other DBH inhibitors such as fusaric acid (FA). FLA-63 and FA produce similar reductions in levels of NE over a range of doses [28]; increased DA levels noted with FLA-63 [29] have not been found even with high doses (100 mg/kg) of FA [18].

One problem with the use of DBH inhibitors is that many (including FLA-57, diethyldithiocarbamate, U-14,624 and FLA-63) have adverse side effects and are lethal at high doses [10]. Peripheral administrations of these drugs result in changes in motor activity [14, 23, 25, 26], which may confound studies examining food intake. The effects of FA with respect to motor and aggressive behaviours have been studied in mice [7]. Although 50 mg/kg produced behavioural deficits, the changes in monoamine levels were different than previously found in rats, suggesting a species difference. Clearly the behavioural effects of these drugs require further examination.

The effects of several doses of FLA-63 and FA on feeding behaviour and motor activity were determined in Experiments 1 and 2. In Experiment 3, restoration of feeding by ventromedial hypothalamic (VMH) NE injections after FLA-63 or FA was investigated. Central administrations of morphine, which elicit feeding (see [16] for a review), were also examined in animals treated with these DBH inhibitors as the endogenous opiates may function as a co-transmitter with NE [27].

EXPERIMENT 1: FLA-63, FA AND LOCOMOTOR ACTIVITY

Method

Two groups of 24 male Wistar rats weighing 250–350 g, obtained from Charles River Canada, were housed individually in 25.4×15.2×15.2 cm cages on a 12 hr light/dark cycle (lights on from 0800 to 2000 hr) and maintained with ad lib food (Purina rat chow) and water except when in the testing apparatus. Motor behaviour was monitored using six automated activity chambers (41.0×50.5×37.0 cm) described previously [4].

FLA-63 (Sigma) was suspended in a 0.5% solution of Tween 80 and water and buffered with NaOH to a pH of 6 before injection. Doses were 1.25, 2.5, 5.0 mg/kg and a 0.5% Tween 80 vehicle. FA (Sigma) was dissolved in 0.9% saline and buffered. Doses were 20, 40, 80 mg/kg and a saline vehicle.

All rats had several hr of exposure to the apparatus prior to testing. Each group (n=6) was tested once, receiving a DBH inhibitor at approximately 1400 hr. Animals were removed from their home cages, injected intraperitoneally, and placed in an activity monitor. One group, and thus one dose of one of the DBH inhibitors, was tested on each day, the order of testing of each drug and dose being randomly assigned to days. Number of beam-crossings was recorded per hr over a 7 hr period.

Results

An analysis of variance revealed that FLA-63 increased activity, $F(3,20)=52.34$, $p<0.001$. Dunnett's post hoc tests showed that 2.50 and 5.00 mg/kg FLA-63 produced higher levels of activity than controls, $d(df=20)=3.82$, $p<0.01$, $d(df=20)=3.45$, $p<0.01$, respectively. Activity varied with time, $F(6,120)=7.41$, $p<0.001$, and the dose effect changed over time, $F(18,120)=1.80$, $p<0.032$ (see Fig. 1). No significant effect of FA was found on activity (see Fig. 1).

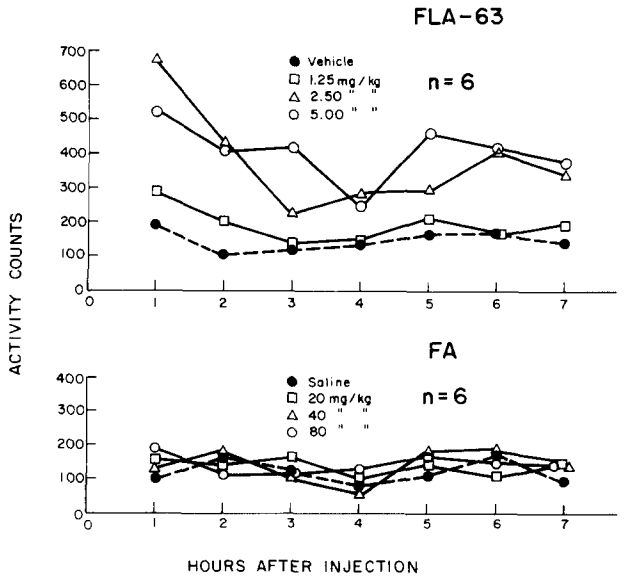

FIG. 1. The effects of IP administration of various doses of the dopamine-β-hydroxylase inhibitors fusaric acid (FA) and FLA-63 on activity, as measured by photobeam crossing counts, are shown over the 7 hr of testing. While FLA-63 increased activity with higher doses, FA had no significant effect.

Discussion

Though the increase in activity found with FLA-63 is consistent with the findings of Lemmer and Berger [14], the present increases were evident with 5.0 mg/kg FLA-63 as opposed to the 40 mg/kg they used. It has been suggested that FLA-63 may increase activity because of its effects on DA [29]. FLA-63 has been shown to cause both a buildup of DA [2, 6, 23, 28] and increase its release, as measured by the catecholamine metabolites normetanephrine and methoxytyramine [1], thus FLA-63 could produce a stimulant effect [14].

That FA had no significant effect on activity is difficult to interpret in light of the effects of FLA-63. Though doses both higher and lower than those used by Diringer *et al.* [7] were studied here, the marked activity deficits they reported in mice were not found. Species differences could have been responsible for this discrepancy. For rats, the effects on activity of FA appear to be quite different from those of FLA-63. This would be consistent with the above hypothesis concerning DA involvement since DA levels have been reported to be altered relatively little with FA when compared to FLA-63 at doses which reduce NE to a similar degree [28].

EXPERIMENT 2: FLA-63, FA AND FOOD INTAKE

Method

48 male Wistar rats weighing 250–350 g were maintained as described in Experiment 1. Testing was carried out in cages identical to home cages. Water was available ad lib. Metal lids were placed over the cages and plastic sheeting underneath to facilitate the gathering of uneaten food which was weighed to the nearest 0.1 g. The drugs and doses used in Experiment 1 were employed.

Four groups (n=5) received FA and 4 groups (n=7) FLA-63. Animals were removed from their cages, injected with the appropriate dose of drug at approximately 1400 hr

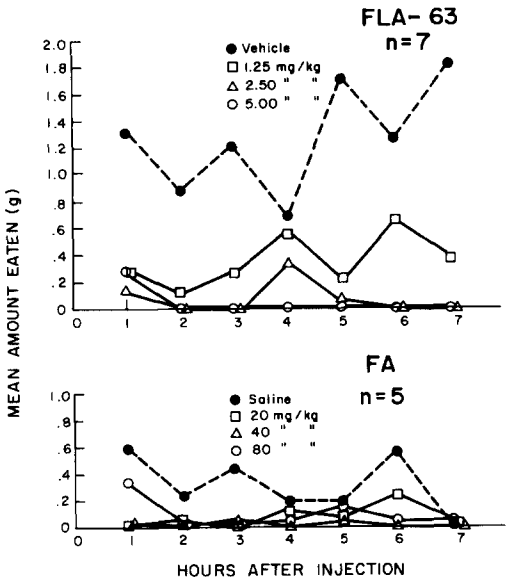

FIG. 2. The effects of IP administration of various doses of the dopamine-β-hydroxylase inhibitors fusaric acid (FA) and FLA-63 on food intake over the 7 hr of testing. Both drugs significantly reduced food intake at all doses.

and placed in the testing cages with pre-weighed food. Once each hour, for 7 hr, the remaining food was removed, weighed and replaced with fresh food. All FA animals were tested on the same day; FLA-63 animals were tested on a separate day.

Results

FLA-63 produced a decrease in feeding. This was confirmed in a dose × time analysis of variance that revealed a significant effect of dose, $F(3,24)=3.53$, $p<0.030$. FA also resulted in significant reductions in food intake, $F(3,16)=3.94$, $p<0.028$. Dunnett's post hoc tests showed that each dose of FLA-63 and FA reduced food intake compared to controls, $d(df=24)=2.21$, 2.84, 2.93, $p<0.05$ for 1.25, 2.50 and 5.00 mg/kg FLA-63, and $d(df=16)=2.44$, 3.85, 2.79, $p<0.05$ for 20, 40 and 80 mg/kg FA (see Fig. 2).

Discussion

FLA-63 and FA were found to decrease food intake over a range of doses. The dose of FLA-63 (1.25 mg/kg) which produced no significant effects on activity resulted in decreases in food intake, as did doses which produced increases in activity. Furthermore, reductions in food intake were noted with doses of FA which produced no significant effects on activity. Therefore, there would seem to be no correlation between the effects of DBH inhibition on food intake and the level of locomotor activity. Thus a motor deficit confound would seem unlikely. The results suggest that inhibition of NE synthesis produced reductions in food intake.

EXPERIMENT 3: CENTRAL REINSTATEMENT OF FEEDING REDUCED BY FLA-63 OR FA

The purpose of this experiment was to test the possibility that the reduction in feeding produced by DBH inhibitors could be reversed by intrahypothalamic injections of NE. On the basis of the previous experiment, 40 mg/kg FA and 5.0 mg/kg FLA-63 were selected as appropriate doses for continued study with respect to feeding. As opiates have been shown to modulate appetite [16], the effects of central injections of morphine also were tested.

Method

28 male Wistar rats weighing 200–250 g were maintained as described in Experiment 1, with the exception that they were food deprived for 24 hr prior to surgery. Rats were anaesthetized with 50 mg/kg Sodium Pentobarbitol and unilateral guide cannulae (modified 23 gauge needles) were stereotaxically implanted such that the tips extended to 0.2 mm above the VMH; the coordinates were 0.4 mm anterior to bregma, 0.5 mm lateral to the midline and 8.3 mm below the dura mater with the incisor bar set at 5 mm above the interaural line [19]. Cannulae were kept sealed when injections were not being made with stainless steel obturator pins, secured with a small quantity of silicone sealant.

Intrahypothalamic injections were made through modified 30 gauge needles which fit inside the guide cannula and extended 0.4 mm beyond its tip. Centrally administered drugs were always given in 0.5 μl of 0.9% saline using a 5.0 μl Hamilton microsyringe. The doses were 60.0 nmoles of NE in the FLA-63 pre-treated animals and 30 nmoles of NE in the FA pre-treated animals, 5.3 nmoles of morphine and a saline vehicle. The doses of peripherally administered drugs were 5.0 mg/kg of FLA-63 and 40.0 mg/kg of FA.

Testing was carried out on every third day in the apparatus described in Experiment 2 with food available ad lib. Peripheral injections were made 4 hr prior to central injections. Food intake was measured for the 3 hr following central injections.

For the FLA-63 experiment, animals were pre-treated with either FLA-63 (n=6) or its vehicle (n=6) prior to each of 3 central injections. Each animal received central injections of NE, morphine and saline on separate days with the order of administration counterbalanced.

The FA experiment involved 11 animals that were tested six times. Animals were pre-treated with either FA (n=5) or its vehicle (n=6) prior to the first 3 injections; after this a reversal was conducted where previously FA pre-treated animals were pre-treated with the vehicle and vice versa. The 3 central administrations were NE, morphine and saline, given on separate days with the order of administration counterbalanced for each of the two peripheral injection cycles.

To verify cannulae placements, the rats were killed with overdoses of sodium pentobarbitol, exsanguinated with 0.9% saline followed with 10.0% formalin and their brains were removed. Brains were sliced at 50 μm, mounted and stained with methionine.

Results

Cannula placements, as shown in Fig. 3, were in the VMH.

Food intake of the peripherally administered vehicle/FLA-63 group is shown in Fig. 4 and was analyzed using a 3-way mixed design analysis of variance with peripheral drug pre-treatment, central drug and time as the variables analyzed, the latter 2 being repeated measures. Results revealed a significant effect of peripheral drug, with FLA-63 significantly reducing food intake, $F(1,10)=15.93$, $p<0.003$,

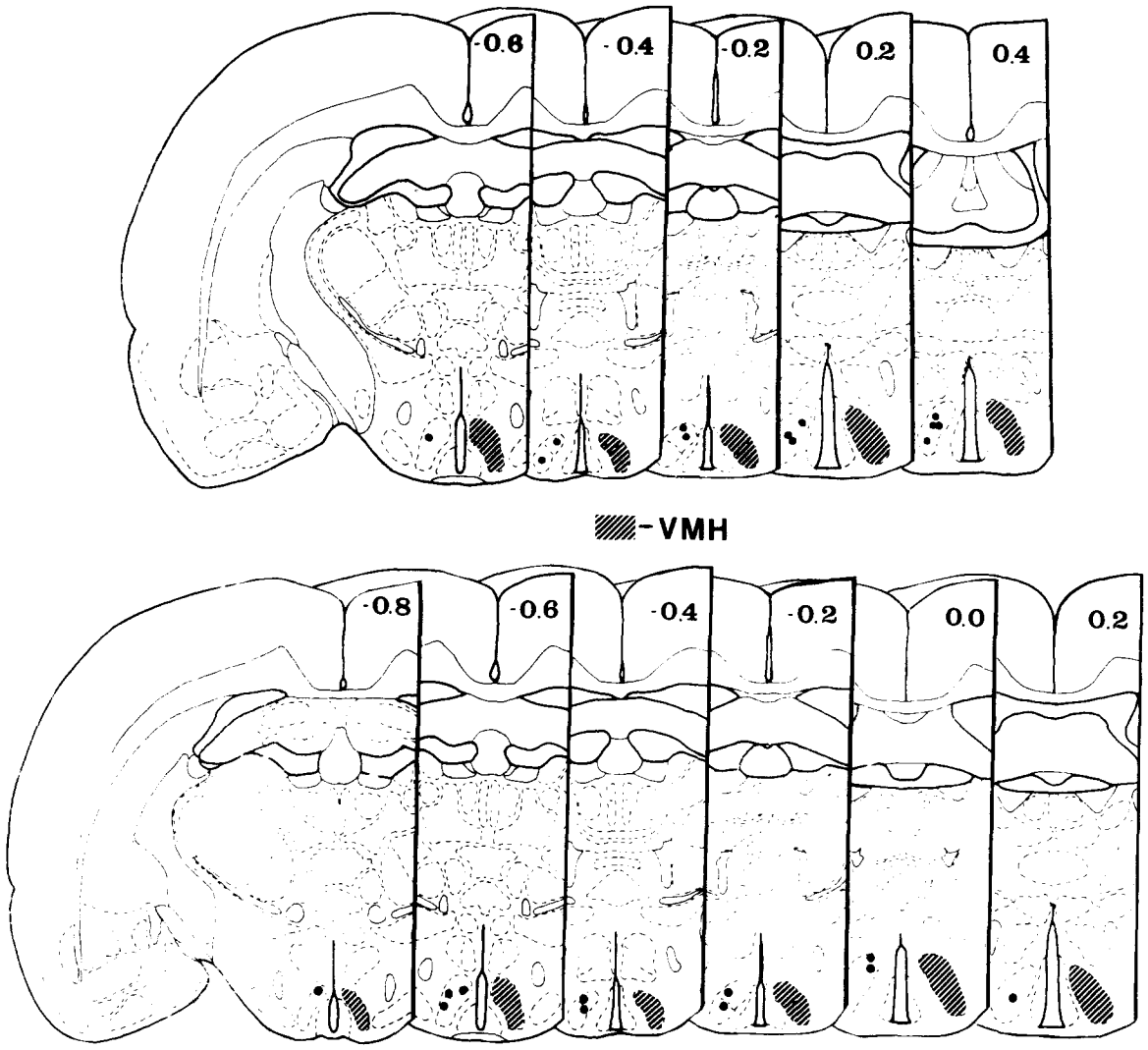

FIG. 3. Schematic diagrams showing cannula tip placements (filled circles) in the ventromedial hypothalamus (VMH) of the 12 rats used in the FLA-63/central injection experiment (top sections) and the 11 rats used in the Fusaric Acid/central injection experiment (bottom sections). Shaded areas indicate the VMH (adapted from [16]).

a significant effect of central drug, $F(2,10)=11.29$, $p<0.001$, and a significant central drug × time interaction, $F(4,40)=7.74$, $p<0.001$; none of the remaining main effects or interactions were significant. Post hoc Dunnett's tests of pairwise comparisons in the vehicle pre-treated animals revealed a significant effect of NE relative to the central saline controls in the first hour following injection, $d(df=10)=2.90$, $p<0.025$, and in the third hour, $d(df=10)=2.32$, $p<0.05$. Following treatment with FLA-63, NE treated animals ate significantly more than their central saline controls in the first hour after central injection, $d(df=12)=3.051$, $p<0.01$. Morphine treatment resulted in no significant increases in feeding (see Fig. 4).

Food intake of the peripherally administered saline/FA group is shown in Fig. 5 and was analyzed with a 3-way repeated measures analysis of variance with peripheral drug pre-treatment, central drug and time as the variables analyzed. Results revealed a significant effect of peripheral drug, with FA reducing food intake, $F(1,10)=6.33$, $p<0.029$, a significant effect of central drug, $F(2,20)=4.60$, $p<0.022$, and a time × drug interaction, $F(4,40)=10.28$, $p<0.001$. None of the remaining main effects or interactions was significant. Post hoc tests for the saline pre-treatment condition showed that central NE treatment resulted in increased feeding in the first hour following injection, $d(df=20)=2.16$, $p<0.05$; morphine treatment produced no significant effect. Similarly for the FA pre-treatment condition, food intake was increased with NE treatment in the first hour following injection, $d(df=20)=5.84$, $p<0.005$; morphine, in this case, produced a significant effect in the third hour, $d(df=20)=3.98$, $p<0.005$ (see Fig. 5).

Discussion

The findings of Experiment 2 were replicated in Experiment 3: FLA-63 and FA reliably reduced food intake. No significant 3-way interactions or peripheral × central drug interactions were found, suggesting that the effects of NE

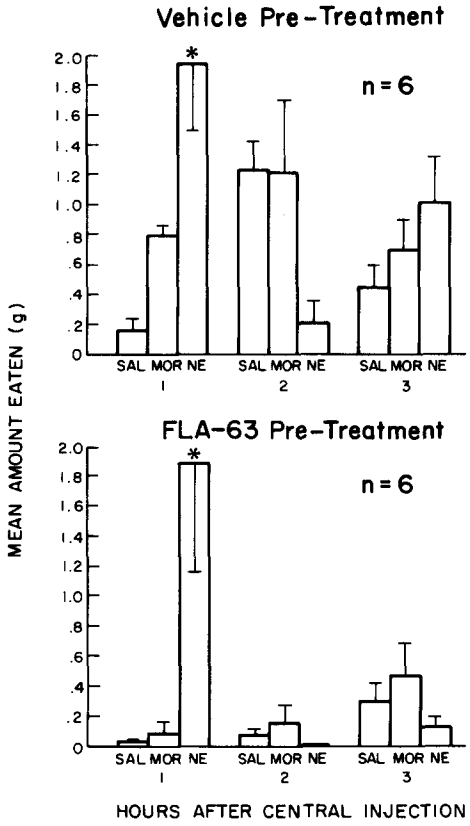

FIG. 4. The mean (±s.e.m.) amount eaten following intrahypothalamic injections of norepinephrine (NE), morphine (MOR) and saline (SAL) administered 4 hr after injection of peripheral vehicle or peripheral FLA-63 over the 3 hr of testing. NE injection resulted in increased feeding in the first hr following central injection in both peripheral conditions. MOR had no significant effect. Asterisk indicates significant difference from control, $p<0.05$.

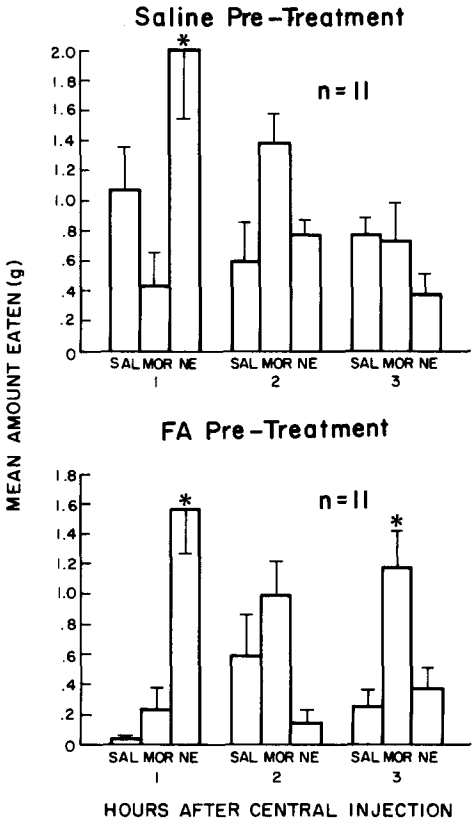

FIG. 5. The mean (±s.e.m.) amount eaten following intrahypothalamic injections of norepinephrine (NE), morphine (MOR) and saline (SAL) administered 4 hr after injection of peripheral saline or peripheral FA over the 3 hr of testing. NE injection resulted in increased feeding in the first hr following central injection in both peripheral conditions. Morphine injection resulted in feeding only in the third hr under FA pre-treatment. Asterisks indicate significant difference from control, $p<0.05$.

and morphine on feeding did not vary with the peripheral manipulation. Thus, the effects of NE or morphine on a DBH-inhibited animal are probably additive rather that multiplicative.

Central administrations of NE or morphine reinstated feeding reduced by inhibition of DBH, providing further support for the contention that the reduction in feeding was related to altered neurotransmitter synthesis rather than nonspecific impairment. Thus, the animals were capable of eating.

Central injection of NE but not saline or morphine produced an immediate and pronounced reinstatement of feeding. This differential effect suggests that NE was mediating the feeding response. The finding that NE-induced feeding was of rapid onset and short duration is consistent with many previous reports [13, 20, 24]. These data support the hypothesis that NE in the VMH is directly involved in the effect of FLA-63 and FA on feeding.

It is interesting to note that central morphine also produced feeding, though in a delayed manner and only in the FA condition. This delay has been noted elsewhere [24] and is probably not an effect which is peculiar to DBH inhibited animals.

CONCLUSIONS

Doses of FA which produced no significant effect on locomotor activity resulted in deficits in food intake; further, reinstatement of feeding was possible with injection of NE or morphine into the VMH. FLA-63, on the other hand, produced significant increases in activity at higher doses while producing deficits in food intake that could be reinstated by intrahypothalamic NE. These findings suggest several conclusions.

DBH inhibitors appear to produce effects on feeding which are not due to a general impairment. Further, NE in the hypothalamus appears to be directly involved in the control of feeding. The immediate and large effect of NE makes it unlikely that its action was at a site other than the hypothalamus, although it is difficult to be certain that other hypothalamic nuclei close to the VMH were not involved.

Even though food intake has been reduced by inhibition of DBH, central NE rapidly produced feeding while morphine had no significant effect. This further suggests that the effect of DBH inhibition on feeding was due to a reduction in the synthesis of NE. However, since the effects on feeding of central NE were similar in the vehicle and DBH-inhibited

rats, it is possible that the NE effect was separate from the DBH effect. For example, it has been suggested that the increase in DA noted with these drugs results in an amphetamine-like decrease in food intake [29]; from this point of view, the effects of central NE on feeding did not represent a reversal of the DBH effect. Thus, conclusions with regard to NE reinstatement of DBH-inhibitor-reduced feeding should be reserved in nature.

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