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BRIEF COMMUNICATION

Behavioral Depression: Thyroid Interactions with Norepinephrine-Depleting Drugs[1]

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DAVENPORT, J. W., R. S. HENNIES, J. C. CAREY AND S. B. BISHOP. *Behavioral depression: thyroid interactions with norepinephrine-depleting drugs.* PHARMAC. BIOCHEM. BEHAV. 5(1) 87–89, 1976. – Temporary suppression of rats' bar pressing, activity, and feeding by the dopamine β-hydroxylase inhibitor FLA-63 was synergistically potentiated by triiodothyronine (T_3) treatment. The increased severity and duration of behavioral depression from the combination of mildly-depressing doses of FLA-63 (10 mg/kg, SC) and T_3 (200 μg/kg, SC, 4X) was most marked 36–72 hr after FLA-63 and closely resembled the depressive syndrome produced by higher (30–90 mg/kg) doses of FLA-63 alone in timing and specific behavioral features; these depressions were more likely due to toxicity than to depletion of brain norepinephrine. T_3 did not potentiate behavioral depression induced by diethyldithiocarbamate (25 mg/kg, SC). This pattern of findings suggested an interpretation of the T_3-FLA-63 synergism in terms of increased FLA-63 toxicity in hyperthyroidism.

Hyperthyroidism Norepinephrine FLA-63 Diethyldithiocarbamate Bar pressing Activity Feeding Behavioral depression

HOPING to clarify further the role of central thyroid-catecholamine interactions in behavior, we have studied how triiodothyronine (T_3) modifies behavioral depressions in rats resulting from drugs which selectively deplete norepinephrine (NE) by inhibition of dopamine-β-hydroxylase (DBH), the enzyme which converts dopamine (DA) to NE. In several experiments we have used the DBH inhibitors FLA-63 [bis (4-methyl-1-homopiperazinyl-thiocarbonyl) disulfide] and diethyldithiocarbamate (DDC). Both of these drugs rapidly deplete brain NE [3, 5, 7] and concurrently suppress gross activity, food intake, food-reinforced bar pressing, and shuttle box avoidance performance [5,7, unpublished data] in rodents. Three surprising findings have emerged in our studies: (1) behavioral depression from FLA-63 was greatly potentiated by T_3 treatment; (2) in sharp contrast, T_3 did not affect DDC-induced depression; and (3) when administered alone, the two DBH inhibitors also clearly differed in the nature and time course of behavioral depression which they induced.

Our general procedure was to train adult male Holtzman rats, usually experimentally naive and having no previous drug experience, to nearly asymptotic performance in daily 20-min fixed ratio (FR 20) barpessing sessions in modified Gerbrands operant conditioning chambers and 1-hr activity sessions in standard Wahmann running wheels, and to continue this testing for several days after injections. Since the bar pressing was food reinforced, the rats were maintained at 80% of free feeding weight by means of restricted daily feedings usually in the afternoon, 18–20 hr prior to behavioral testing. Injections were given in the early afternoon, between the daily testing and feeding times. In some studies extra bar pressing and activity sessions were given in the afternoons and evenings of postinjection days, and these tests were supplemented with measures of food and water intake and observations of homecage behaviors. The studies involved various doses and combinations of T_3, (3, 3, 5-triiodo-l-thyronine, ICN Nutritional Biochemicals), dissolved in saline, FLA-63 (Regis), dissolved in 50% ethanol, and DDC (sodium diethyldithiocarbamate, Sigma), dissolved in distilled water. All injections were subcutaneous.

Three experiments (total N = 94) agreed in showing considerably greater behavioral depression in rats receiving combinations of T_3 and FLA-63 than in rats receiving either agent alone. One of these (Fig. 1) revealed a T_3-FLA-63 interaction in bar pressing that was synergistic, in the strict sense that the severity of depression from the combination exceeded the sum of the mildly suppressive effects of the two agents acting independently. In this

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study, we used a 2 × 2 factorial design in which the combined group received 200 μg/kg T_3 injections on four consecutive days and a single injection of 10 mg/kg FLA-63 on the second of these days, while three comparison groups received four T_3 and one alcohol-vehicle injection (T_3 Alone), four saline and one FLA-63 injection (FLA-63 Alone), or four saline and one alcohol-vehicle injection (Control). Two additional combined groups which were given higher (670 μg/kg) and lower (67 μg/kg) T_3 doses with the same FLA-63 dose were injected and tested concurrently.

All 18 rats in the three combined groups displayed nearly total cessation of barpressing in the second and third test sessions (40 and 62 hr) after the FLA-63 injection, in contrastto maximal decrements averaging 25% (40 hr) and 40% (62 hr) in the FLA-63-Alone and T_3-Alone groups, respectively. Running wheel activity (see lower panel, Fig. 1) and food and water intake were also severely depressed in the combined groups during this post-injection period. In their homecages, these rats slept excessively and usually assumed a lying-down posture when awake, but displayed essentially normal eye-opening, posture, and locomotor behavior when stimulated during weighings and other handling. There was little difference among the three combined groups in severity of depression during this period, but recovery was dose-dependent; four rats in the 670 μg/kg T_3-plus-FLA-63 group failed to return to normal (T_3-Alone) barpressing and activity levels and two of these died near the end of testing.

An interesting hypothesis, suggested by these results, by reports of increased central catecholamine turnover in hyperthyroid rodents [2], and by Stein and Wise's [6] theorizing, is that the combination of T_3-increased DA synthesis and blockade of DA-to-NE conversion by a DBH inhibitor could engender an excessive endogenous formation of 6-hydroxydopamine or other neurotoxic DA byproducts, with sufficient resulting damage to catecholaminergic neurons to produce unusually severe and prolonged behavioral depression. This possibility was largely negated by a further group of factorial-design studies (total N = 72) which showed that suppression of barpessing and activity by DDC (25 mg/kg) was neither potentiated nor ameliorated by T_3 (10–150 μg/kg, 3X) at any point within three days after the DDC injection. These T_3-DDC studies also contradicted another possible interpretation of the T_3-and-FLA-63 results - that the synergistic behavioral effect may have resulted from the summation of mild NE-depleting effects of thyroid treatment [2] and larger NE depletions from DBH inhibition.

Additional studies, on the depressive effects of FLA-63 and DDC alone, led us to question whether the synergistic interaction of T_3 and FLA-63 involved any central thyroid catecholamine interactions at all. In a dose-response study of FLA-63 (N = 22), behavioral testing at 2, 9, 21, 33, 45, 69, 93, and 117 hr after single subcutaneous injections of 0, 7.5, 15, 30, 60, or 90 mg/kg produced the family of barpressing curves in Fig. 2. For each of the five FLA-63 groups there was a characterisitc curve showing an abrupt decrement in barpressing 2 hr postinjection and partial recovery within the first 24 hr (somewhat later in the 90 mg/kg group), followed by a second depression of responding during the second postinjection day and full recovery thereafter. This pattern and the clear dose-response relationship shown in Fig. 2 appeared in activity and feeding behavior as well as in FR 20 barpressing.

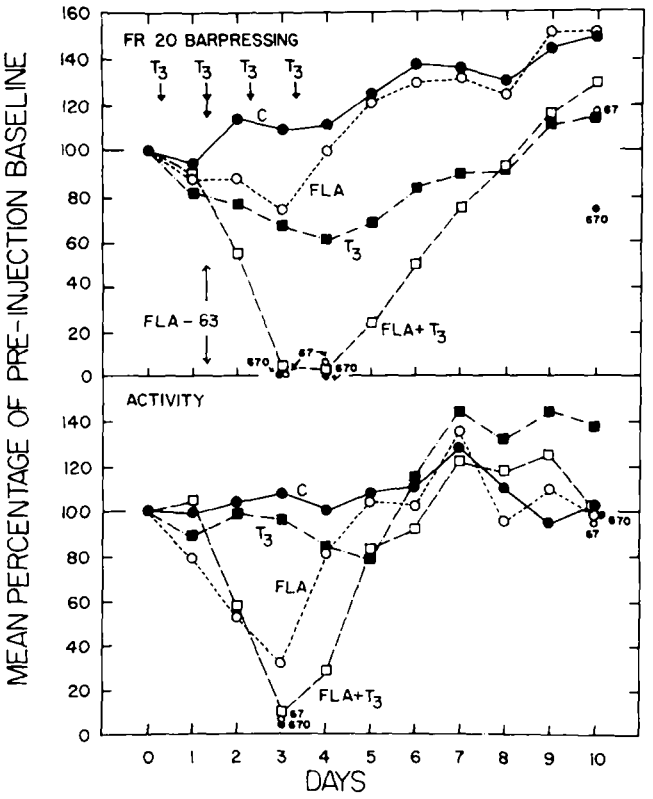

FIG. 1. Interaction of T_3 and FLA-63 in barpressing (upper panel) and running-wheel activity (lower panel). Curves for the Control (C), FLA-63-Alone (FLA), 200 μg/kg T_3-Alone (T_3), and 200 μg/kg T_3-plus-FLA-63 (FLA + T_3) groups are shown for the entire 10-day postinjection period. Mean values for the 670 (small filled circles) and 67 (small unfilled circles) μg/kg T_3-plus-FLA-63 groups are shown for Days 3, 4, and 10. N = 6/group.

Another 27 rats, which received single injections of 25, 50, 100, 200, or 300 mg/kg DDC, also showed dose-related barpressing depressions below vehicle-injected controls within the first 12 hr postinjection, but all recovered monotonically therafter; i.e., no second depression occurred as with FLA-63. In general, the severity and time course of DDC-induced depression and its recovery corresponded well with data in the literature on NE depletion from DDC in rodents.

With FLA-63, we did not find such a correspondence after the first 24 hr postinjection. In 18 additional rats which were given either 30 mg/kg FLA-63 or the alcohol vehicle and which showed the same performance as the O and 30 mg/kg groups in Fig. 2 prior to sacrifice, whole-brain NE content was assayed at 7, 24, and 48 hr postinjection by Endocrine Laboratories, Madison, Wisconsin, using the fluorometric trihydroxyindole method of Miller *et al.* [4]. Compared to vehicle-injected rats' NE content (mean, 0.45 μg/g), the drug-treated rats were depleted by averages of 42.8% at 7 hr, 27.2% at 24 hr, and not at all (102.7% of normal) at 48 hr postinjection. (Brain DA and serotonin values, also assayed, were essentially normal at all time points.) Thus, while some correlation of behavioral depression and NE-depletion from FLA-63 was apparent within the first 24 hr postinjection, in line with most previous research, the second depression from FLA-63

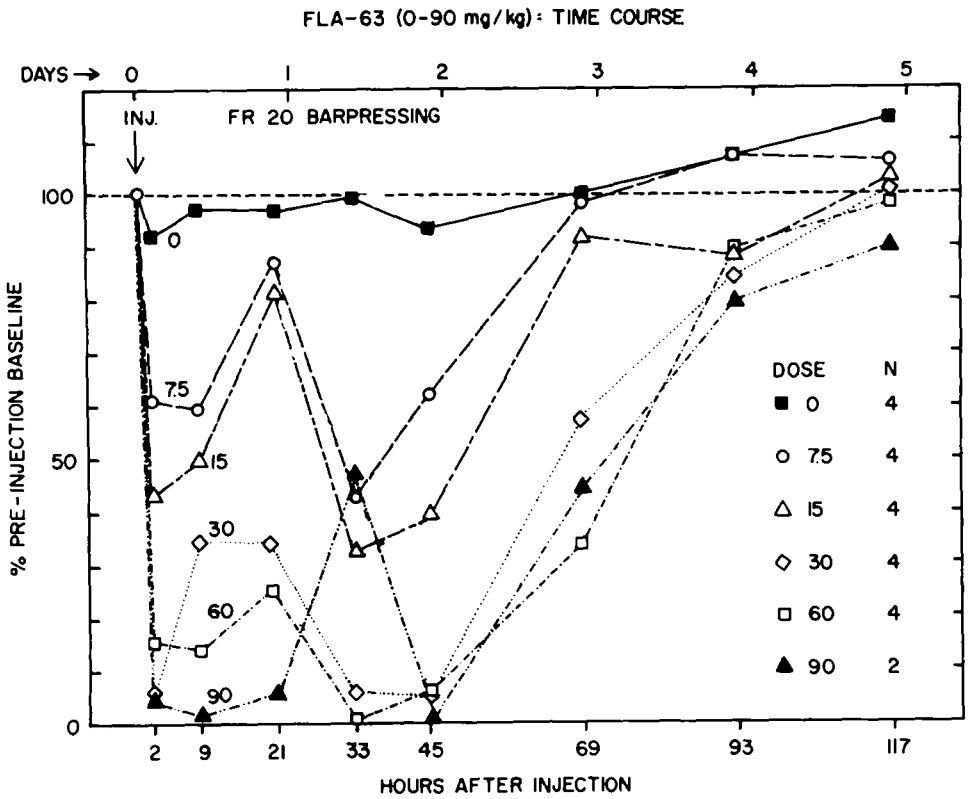

FIG. 2. Effects of FLA-63 alone in single subcutaneous doses up to 90 mg/kg on FR 20 bar pressing over 5 postinjection days. Note the partial recovery of bar pressing at 21 hr prior to a second depressive phase at 33–45 hr in the 7.5–60 mg/kg groups and the similar pattern which occurred later in the 90 mg/kg rats.

which occurred following partial recovery was associated with normal or near-normal NE content.

The depressive syndrome produced by our higher (60–90 mg/kg) doses of FLA-63 alone during the 36–72 hr postinjection period (Fig. 2) was highly similar to the severe depression produced by combining 10 mg/kg FLA-63 with various T_3 doses (Fig. 1), both in terms of timing and specific behavioral features. Very likely, considering the discrepancy we found between this syndrome and the normal NE values 48 hr after 30 mg/kg FLA-63 alone, the second depression represents toxicity from subcutaneous FLA-63 in the form of temporary systemic illness. Additional pilot work and unpublished reports from other laboratories indicate that this drug is unusually toxic when administered by other routes, with doses as low as 25 mg/kg (IP), 5 μg (intraventricular), and 100 mg/kg (PO) proving to be lethal in rats.

Increased toxicity of many centrally acting drugs (e.g. amphetamine, imipramine, pentobarbital, morphine) in hyperthyroid animals has been amply demonstrated [1]. The general pattern revealed here strongly suggests that our initial finding – potentiation by T_3 of FLA-63-induced behavioral depression – represents another instance of such enhanced toxicity. While more detailed toxicological information on FLA-63 is needed, there is already enough evidence to indicate some severe limitations in the usefulness of this drug for investigating the role of brain catecholamines in behavioral phenomena.

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