Pharmacology Biochemistry and Behavior, Vol. 45, pp. 495–500, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Stress and Dexfenfluramine: Effects on the Immune Response and Energy Balance in the Rat

ANNE-MARIE SOUQUET AND MARC FANTINO[1]

Laboratoire de Neurobiologie des Régulations Alimentaires,
Département de Physiologie Humaine et Expérimentale, Faculté de Médecine/Université de Bourgogne,
7 Boulevard Jeanne d'ARC, F-21033 Dijon, Cédex, France

Received 21 September 1992

SOUQUET, A. M. AND M. FANTINO. *Stress and dexfenfluramine: Effects on the immune response and energy balance in the rat.* PHARMACOL BIOCHEM BEHAV 45(2) 495–500, 1993.—The effects of stress, dexfenfluramine (*d*-Fen), and a combination of both were investigated on ingestive behavior, body weight, and the humoral immune response in the rat. Three-hundred and 84 male Sprague-Dawley rats were split into four groups of 96 animals. In a balanced design, each group was submitted or not to repeated intense stress for 20 consecutive days. Animals were also treated with 5 mg/kg/day *d*-Fen (IP, 1 ml/kg) or an equal volume of placebo (saline) for 28 days. The humoral immune response of rats to sheep red blood cells (50% solution, 1 ml IP at day 0) was assessed from the antibody titer on days 4, 8, 12, 16, 20, and 28. Antibodies were assayed by direct hemagglutination and by the Coombs' test. Plasma corticosterone was also measured on days 0 and 12. The effects of stress and *d*-Fen on ingestive behavior and body weight were consistent with previously published results. In addition, rats treated with *d*-Fen had a significantly reduced body weight (−20 g) 5 weeks after the end of the treatment, whereas the loss in body weight induced by stress had totally disappeared. Stress did not decrease animals' immune response despite a massive corticosterone secretion on day 0, with a marked response lasting for at least 12 days. *d*-Fen reduced the corticosterone levels determined on day 12. Antibody production was slightly but significantly reduced in rats receiving *d*-Fen. These results indicate that the humoral immune response may be independent of corticosterone secretion, explaining why the *d*-Fen-induced reduction in the corticosterone response to stress did not compensate for the effect of this drug on antibody production.

Body weight Immune response Food intake Stress Dexfenfluramine

IT has been suggested that serotonin [5-hydroxytryptamine (5-HT)] inhibits the immune response. For example, lesions of the raphe nucleus in the rat reduce brain 5-HT content and increase the immune response to bovine serum albumin (16). In the mouse, administration of 5-HT or its precursor 5-hydroxytryptophan (5-HTP) decreases the humoral and cellular responses to sheep red blood cells (5). To explain these effects, these authors proposed a central interaction between 5-HT and the immune system, mediated by the hypothalamic-pituitary-adrenal axis. Serotonin acts on the three main levels of this axis: It increases hypothalamic secretion of corticotropin-releasing factor (CRF) (11,21,22); serotonin agonists directly stimulate adrenocorticotropin (ACTH) secretion independently of their effect on endogenous CRF (10,48); they stimulate adrenal-glucocorticoid secretion (1,10). A direct peripheral action of 5-HT on immunocompetent cells has also been demonstrated: In vitro, raised concentrations of this mediator reduce proliferation of lymphocytes after stimulation by concanavalin A or lipopolysaccharides (39).

These observations indicate that 5-HT agonists used therapeutically could depress the immune response. Indeed *d*-fenfluramine (*d*-Fen), a 5-HT agonist widely used in the treatment of obesity, induces an acute increase in plasma corticosterone (7), and this corticosterone response is prevented by electrolytic or chemical lesion of the raphe nucleus, which is suggestive of a central involvement of *d*-Fen on the hypothalamic-pituitary-adrenal axis (44). In addition to CRF release, *d*-Fen also acts directly on the pituitary and adrenal glands, stimulating secretion of ACTH and corticosterone (29).

However, chronic administration of *d*-Fen might also stimulate the immune response, especially in stressful situations. Indeed, daily administration of *d*-Fen, (10 mg/kg/day, IP) for 12 days has been found to attenuate the effects of a metabolic stress, reducing the duration and amplitude of the hyper-

[1] To whom requests for reprints should be addressed.

corticosteronemia it induces (7). Moreover, *d*-Fen reduces tail-pinch-induced hyperphagia (2,41,47). Finally, in the mouse selective depletion of brain 5-HT content by a specific neurotoxin (5,7-dihydroxytryptamine, after pretreatment with desipramine) did not affect antibody production (24).

So, the interaction of *d*-Fen and stress and the role of glucocorticoid secretion in physiological responses such as feeding, drinking, body weight, and the immune response is not well understood. In the present study, we evaluated ingestive behavior and the immune response of rats to an injection of sheep red blood cells (SRBCs) while animals were subjected to repeated stress and chronically treated with *d*-Fen or a placebo.

METHOD

Animals and Housing Conditions

Three-hundred and 84 male Sprague-Dawley rats from Iffa Credo France, initially weighing (at day 0) 263–301 g, were split into four groups of 96 animals, housed initially 8 per cage in two different rooms at 25 ± 1°C under a 12 L : 12 D cycle (light on at 0600 h). They had free access to food and water except during the stress sessions.

Experimental Procedure

The four groups were: nonstressed/vehicle-treated; stressed/vehicle-treated; nonstressed/*d*-Fen-treated; stressed/*d*-Fen-treated. To prevent any risk of interference between stressed and nonstressed rats (37), the groups were placed in different rooms. On day zero, after the first *d*-Fen or placebo administration rats were immunized by an IP injection of 1 ml of a 2% solution of SRBCs dissolved in saline (stock solution at 50%, Biomerieux, France). From days 0–28, rats of the two *d*-Fen-treated groups received at 0830 h a daily IP 5-mg/kg injection of *d*-Fen HCl (Servier, Neuilly, France) dissolved in saline (5 mg/ml) 0.5 h before the stress procedure. Vehicle-treated rats served as controls and received an isovolumic injection of saline. From days 0–20, stressed rats were subjected to three types of stresses in turn: Cold stress consisted of immersion by groups of 6 rats in cold water (150–200 mm deep) at +8°C for 5 min; heat stress consisted of placing 10 rats for 20 min in a heat box with a floor temperature at 44 ± 1°C (close to the thermal pain threshold) and with surrounding temperature at 33 ± 1°C; rats could avoid the hot floor by climbing onto an insulated surface, from where they were regularly brushed off by a rotating bar (this procedure introduced competition between rats for access to the cool surface); immobilization stress consisted of maintaining rats in cylindrical boxes (65 mm diameter) for 60 min at room temperature. On odd days, rats were subjected to the heat and immobilization stresses. On even days, rats were subjected to the cold stress.

Body weight, food intake (after collection of spillage), and water intake were recorded daily before the stress sessions, at 0830 h. To avoid further stress induced by blood sampling, blood samples were obtained by decapitation of 12 rats out of each group on days 0, 4, 8, 16, 20, and 28 for assay of antibody titer, hematocrit, and corticosterone. To ensure that all animals were free of SRBC antibodies, the first blood samples were taken on day 0 before injection of the antigens. Because corticosterone levels reach a peak 30 min after the start of the stress and remain high for the following 4 h (33,35), to obtain optimal values for the plasma corticosterone levels animals were killed 1 h after *d*-Fen or vehicle injection and for stressed animals about 45 min after the cold stress.

The blood samples were immediately centrifuged for 15 min at 2,500 rpm, then decanted and stored at −20°C until analysis. Hemagglutination titers were determined on microtiter plates, and the highest dilution producing hemagglutination was taken as the antibody titer. Two semiquantitative methods were employed: direct agglutination and the indirect method of Coombs. The minimum dilution was 1/10 for the Coombs method and 1/5 for the direct agglutination method; subsequent dilutions were 1/2. Plasma corticosterone levels were measured on days 0 and 12 with a radioimmunological kit using a dog transcortin serum without extraction of standard or plasma (kit ^{3}H-cortisol, CPB BioMérieux, France). The sensitivity of the assay was 100 pg/tube. The intra- and interassay coefficients of variation were 4.5 and 13%, respectively. Cross-reactivities were: corticosterone 19%, progesterone 26%, 11-deoxycorticosterone 31%, 11-deoxycortisol 17%, and hydroxyprogesterone 92%.

Statistical Analysis

The data for body weight, food intake, drinking, and corticosterone levels were subjected to a three-way analysis of variance [(ANOVA) drug condition, stress condition, and days], followed by a Newman-Keuls test for intergroup comparisons. A probability value of 0.05 was used as the level of significance. The antibody responses were expressed in a graded manner, the minimum dilution being ranked as 1, and the ranked data of different groups were compared by ANOVA.

RESULTS

Food intake

Both stress and *d*-Fen treatment significantly reduced food intake (Fig. 1) [ANOVA from days 0–20: stress effect, $F(A) = 554.53$, $p < 0.001$; *d*-Fen effect, $F(B) = 345.32$, $p < 0.001$; day effect, $F(C) = 26.01$, $p < 0.001$]. For both the stressed and nonstressed groups, the effect of *d*-Fen on food intake was in line with the usually observed pattern. The maximal effect of the drug occurred on the first day (non-stressed rats: *d*-Fen 15 ± 2 g vs. vehicle 24 ± 2g; stressed rats: *d*-Fen 14 ± 2g vs. vehicle 21 ± 2g), then declined, and by the end of the treatment period (day 28) the intake of *d*-Fen-treated rats had reached that of the control rats. Treated animals did not show any overconsumption after the end of the *d*-Fen treatment. Stress also induced an anorectic effect, and it is worth noting from Fig. 1 that in *d*-Fen treated groups as well as in the placebo groups the reduction of food intake induced by the stress progressively increased during the stress period, an effect confirmed by a significant interaction between stress and time [ANOVA from days 0–20: interaction term between stress and time, $F(AC) = 6.28$, $p < 0.001$]. This stress anorectic effect disappeared immediately after the end of the stress-period. Figure 1 shows the potentiation of the anorectic effects of stress and *d*-Fen on food intake, confirmed by a significant interaction of these two factors [ANOVA, $F(AB) = 44.11$, $p < 0.001$].

Water Intake

Stress and *d*-Fen had opposite effects on drinking behavior: Stress from days 0–20 significantly reduced water intake [stress effect, $F(A) = 162.86$, $p < 0.001$], and its effect in-

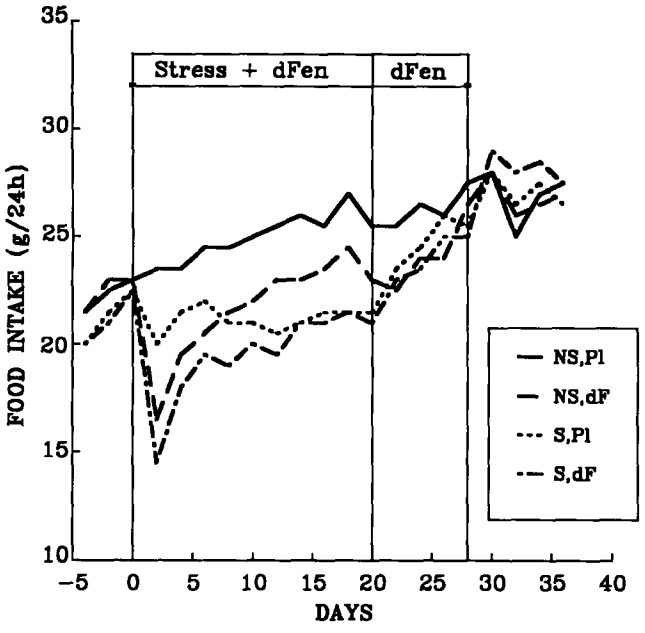

FIG. 1. Mean daily food intake of rats receiving, from days 0–28, 5 mg/kg dexfenfluramine (dF) or placebo (Pl) and submitted (S) or not submitted (NS) to daily stress sessions from days 0–20.

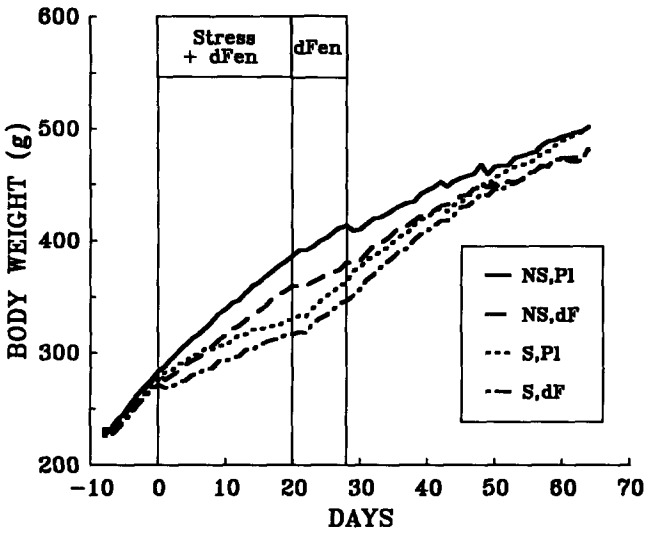

FIG. 2. Body weight in rats receiving, from days 0–28, 5 mg/kg dexfenfluramine (dF) or placebo (Pl) and submitted (S) or not submitted (NS) to daily stress sessions from days 0–20.

creased throughout the stress period [day effect, $F(C) = 8.97, p < 0.001$; interaction, $F(AC) = 4.11, p < 0.001$]; *d*-Fen increased water intake, $F(B) = 187.01, p < 0.001$, with a peak on the first day (nonstressed rats, *d*-Fen 62 ± 16 ml vs. vehicle 37 ± 4 ml; stressed rats, *d*-Fen 54 ± 12 ml vs. vehicle 34 ± 6 ml). This dipsogenic effect persisted until day 28 for the two drug-treated groups.

Body Weight

Both *d*-Fen and stress caused significant weight loss [ANOVA from days 0–20: stress effect, $F(A) = 1579.32$, $p < 0.001$; treatment effect, $F(B) = 667.33, p < 0.001$; day effect, $F(C) = 187.54, p < 0.001$. The day effect results from the normal weight gain of the rat. Figure 2 shows that the weight loss induced by stress alone was larger than the weight reduction caused by *d*-Fen alone. The effect of stress increased with time and was significant from day 12 to the end of the stress period, but disappeared as soon as the stress ended. The stress-*d*-Fen-treated group had the greatest weight reduction, which indicates that the weight-reducing effects of both factors were cumulative. At the end of the stress period (day 20), the mean body weights of the four groups were all significantly different (Newman-Keuls, $p < 0.05$). Five weeks after the end of the stress period, the reduction in body weight induced by stress was no longer significant, and had totally disappeared by the end of the experiment (day 63). The drug effect was sustained throughout the 28-day administration, and *d*-Fen-treated rats still had a significant deficit (−20 g) on the last week of the study [ANOVA from days 57–63, $F(B) = 19.90, p < 0.001$.

Antibody Titers

Assays on day 0 confirmed the lack of previous immunization. Data shown in Figure 3 indicate that stress from days 4–28, in contrast to *d*-Fen treatment, did not affect significantly rats' humoral immune response to SRBC [ANOVA: Coombs—stress effect, $F(A) = 2.34, p = 0.126$; *d*-Fen effect, $F(B) = 5.85, p = 0.016$; days effect, $F(C) = 68.94, p = 0.000$; agglutination—stress effect, $F(A) = 1.54, p = 0.215$; *d*-Fen effect, $F(B) = 3.63, p = 0.056$; day effect, $F(C) = 27.63, p = 0.000$].

Corticosterone

Figure 4 shows that the first session of stress alone resulted in a massive corticosterone secretion. This response was ap-

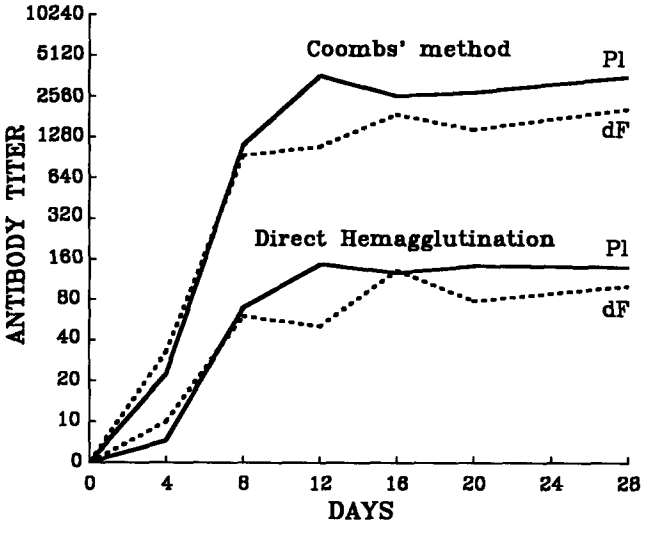

FIG. 3. Mean sheep red blood cell (SRBC) antibody titers, assessed by the Coombs' and direct hemagglutination methods, 0, 4, 8, 12, 16, 20, and 28 days after immunization in rats treated from days 0–28 with 5 mg/kg dexfenfluramine (dF) or placebo (Pl). Because stress did not induce any significant effect on the humoral immune response, stressed and nonstressed groups were pooled.

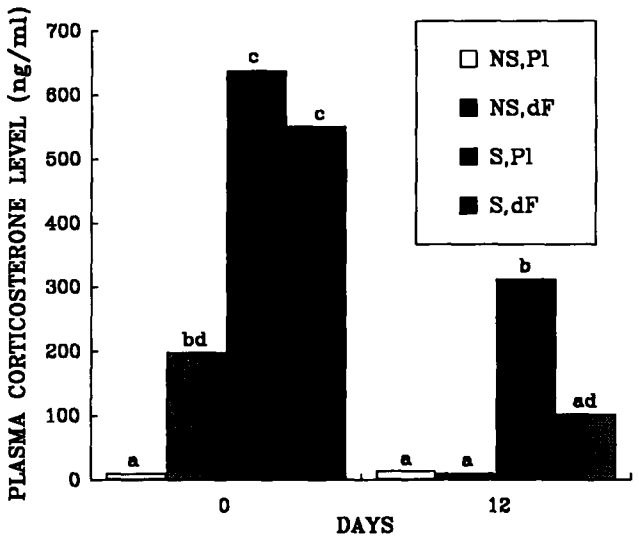

FIG. 4. Plasma corticosterone levels on days 0 and 12 of rats receiving, from days 0–28, 5 mg/kg dexfenfluramine (dF) or placebo (Pl) and submitted (S) or not submitted (NS) to daily stress sessions from days 0–20. Colomns with different letters are significantly different (Newman-Keuls test, $p < 0.05$).

proximately halved on the 12th day of stress. Acute *d*-Fen injection was also followed by a massive corticosterone secretion in nonstressed rats. When associated with stress, *d*-Fen did not amplify this secretion on the first day. Twelve days of *d*-Fen treatment resulted in a significant reduction in the corticosterone response in stressed animals (Newman-Keuls test, $p < 0.05$).

Hematocrit

ANOVA did not detect any effect of stress or *d*-Fen on hematocrit.

DISCUSSION

The present results confirm the well-known effects of stress and *d*-Fen on ingestive behavior, body weight, and corticosterone secretion. They afford a new insight into the relation between these effects and the immune response.

It is known that a mild stress, like tail pinch, stimulates food intake (3,32), whereas an intense stress leads to weight loss (26). In the present study, the intense stress reduced food intake and weight gain. This stress-induced anorexia can also account for the associated reduction in water intake. As shown by numerous workers, dexfenfluramine also reduced food intake and body weight (43). On the other hand, it had a direct dipsogenic action, an effect that Rowland et al. attributed to activation of the peripheral renin-angiotensin system (42). It is worth noting that, although *d*-Fen alone induced a smaller weight loss than stress alone (−7% vs. −15% at day 20), rats that received *d*-Fen still had a significantly lower body weight (−20 g) 5 weeks after the end of the treatment (day 63). This permanent weight reduction after *d*-Fen treatment confirms our previous observations (17) and indicates that the reduction in food intake induced by this drug may involve a different mechanism from the one induced by stress. It may result from the effect of *d*-Fen on body weight regulation, as the drug has been shown to reduce the set-point of this regulation (18).

Stress and *d*-Fen may share a common mechanism of action on food intake, involving CRF and 5-HT. Stress like *d*-Fen increases hypothalamic 5-HT turnover (6), and Shimuzi et al. (46) demonstrated that methysergide, a 5-HT antagonist, prevents the anorexia induced by immobilization in rats. In rats, a release of CRF accompanies the anorexia induced by *d*-Fen (22). Stress activates the hypothalamic-pituitary-adrenocortical axis by a mobilization of CRF (40), and it is well established that CRF has anorectic activity (8,31) that is reversed by the CRF antagonist α-helical CRF (27). Moreover, CRF like 5-HT reduces the preference for carbohydrates (30). Although CRF remains active in hypophysectomized animals, its metabolic effects are attenuated by adrenalectomy, showing that they depend more upon catecholamines than upon corticoids (40).

Acute *d*-Fen raised plasma corticosterone levels in nonstressed rats. Such an increase has been attributed to hypothalamic 5-HT secretion (7,44). This increase was not further augmented in stressed rats as stress-induced corticosterone secretion was likely to be already at a maximum. In contrast, chronic *d*-Fen treatment resulted in a nonsignificant reduction in the corticosterone response to stress. These results are in accordance with previous reports (7,12,44,45) and point to an action of *d*-Fen on the hypothalamic-pituitary-adrenocortical axis. Because of its 5-HT-agonist activity, *d*-Fen facilitates the release of hypothalamic CRF, leading to increased secretion of ACTH and corticosterone.

The most striking finding was that intense and repetitive stress for 20 days did not affect antibody production, although it led to a huge increase in corticosterone secretion. This observation would appear to go against the widely held view that stress has immunosuppressing effects (38). Habituation of rats to stressor may have reduced their response (4,25). However, it has been shown that habituation is specific to the type of stress applied (35) and depends upon duration, intensity, and interval between two sessions (14,34,37). In the present experiments, habituation was minimized by alternative application of stresses of various types and durations. The still large, and even increased, hormonal response after 12 days of stress and the persistence of the stress effect on ingestive behaviors throughout the stress period indicate that habituation was not the cause of the lack of a stress effect on the immune response.

Recent studies indicated that stress preferentially reduces the response of T immunocompetent cells (15). For example, Chayoth et al. (13) found that male rats in an environment of 35°C had a reduced cellular immune response but an increased antitetanus immunoglobulin titer. Calabrese et al. (9) reported a reduced reactivity to stimulation by phytohemagglutinin (PHA) of lymphocytes from stressed rats. A biphasic action of stress on the immune response has also been described. In general, an acute stress lowers the humoral and cellular responses characterizing the alarm phase, while repeated stress results in an opposite effect, that is, an increase in the immune response (23,49). In the present study, the absence of reduced antibody production to stress, despite increased secretion of corticosterone, tends to rule out a role for the corticotropic axis in the humoral response to stress.

In contrast to stress, antibody production was somewhat lower in rats receiving 5 mg/kg *d*-Fen (stressed and nonstressed). This result is not in agreement with the in vitro study of Petrovic et al. (36) showing that the response of B lymphocytes from 21-month-old female rats to lipopolysac-

charides was increased by oral treatment with *d*-Fen (0.6 mg/kg). No change was observed in 5-month-old rats. But, this in vitro study did not take into account interactions existing in the whole organism. On the other hand, a reduced humoral immune response is not incompatible with an increased cellular response and will not necessarily lead to a global reduction in immune defenses. Moreover, it has been shown that the deleterious effect of 5-HT on the immune response results primarily from a direct peripheral action on immunocompetent cells bearing 5-HT receptors (19,20,28). It should be borne in mind that the serotoninergic agonist action of *d*-Fen is essentially at the central level, which could explain its weak inhibiting effect on the peripheral immune response.

In view of the interindividual variations in the antibody titers and large doses of drug employed in the present study, it is unlikely that the slight reduction in antibody titers observed in treated rats would be large enough to impair animals' defences against infections.

ACKNOWLEDGEMENTS

The authors thank Prof. C. Burlet, of INSERM Unit 308, University of Nancy, France, who realised the corticosterone measurements. They also thank Drs. Nathan and Laudignon of Servier, Paris, France, for the generous gift of dexfenfluramine, and Pierre Noirot for excellent technical assistance. This work was supported by grants from the French Ministère de la Recherche et de la Technologie (Programme AGROBIO-Aliments 2002), grants from Institut de Recherche International Servier, and from the University of Burgundy (Dijon, France).

REFERENCES

1. Alper, R. H. Evidence for central and peripheral serotoninergic control of corticosterone secretion in the conscious rat. Neuroendocrinology 51:255–260; 1990.
2. Antelman, S. A.; Caggiula, A. R.; Eichler, A. J.; Lucik, R. R. The importance of stress in assessing the effects of anorectic drugs. Curr. Med. Res. Opin. 6:73–82; 1979.
3. Antelman, S. M.; Szechtman, H. Tail-pinch induces eating in sated rats which appears to depend on nigrostriatal dopamine. Science 189:731–733; 1975.
4. Armario, A.; Lopez-Calderon, A.; Jolin, T.; Balasch, J. Response of anterior pituitary hormones to chronic stress. The specificity of adaptation. Neurosci. Biobehav. Rev. 10:245–250; 1986.
5. Bliznakov, E. G. Serotonin and its precursors as modulators of the immunological responsiveness in mice. J. Med. 11:81–105; 1980.
6. Blundell, J. E. Appetite disturbance and the problems of overweight. Drugs 39:1–19; 1990.
7. Brindley, D. N.; Saxton, J.; Shahidullah, H.; Armstrong, M. Possible relationships between changes in body-weight set-point and stress metabolism after treating rats chronically with D-fenfluramine. Effects of feeding rats acutely with fructose on the metabolism of corticosterone, glucose, fatty acids, glycerol, and triacylglycerol. Biochem. Pharmacol. 34:1265–1271; 1985.
8. Britton, D. R.; Koob, G. F.; Rivier, J.; Vale, W. Intraventricular corticotropin releasing factor enhances behavioral effects of novelty. Life Sci. 31:363–367; 1982.
9. Calabrese, J. R.; Kling, M. A.; Gold, P. W. Alterations in immunocompetence during stress, bereavement, and depression: Focus on neuroendocrine regulation. Am. J. Psychiatry 144:1123–1134; 1987.
10. Calogero, A. E.; Bagdy, G.; Szemeredi, K.; Tartaglia, M. E.; Gold, P. W.; Chrousos, P. Mechanisms of serotonin receptor agonist-induced activation of the hypothalamic-pituitary-adrenal axis in the rat. Endocrinology 126:1888–1894; 1990.
11. Calogero, A. E.; Bernardini, R.; Margioris, A. N.; Bagdy, G.; Gallucci, W. T.; Munson, P. J.; Tamarkin, L.; Tomai, T. P.; Brady, L.; Gold, P. W.; Chrousos, G. P. Effects of serotoninergic agonists and antagonists on corticotropin-releasing hormone secretion by explanted rat hypothalami. Peptides 10:189–200; 1989.
12. Carlton, J.; Rowland, N. E. Long-term actions of D-fenfluramine in two rat models of obesity. II. Responses to stress. Int. J. Obesity 14:31–37; 1990.
13. Chayoth, R.; Christou, N. V.; Nohr, C. W.; Yale, J. F.; Poussier, P.; Grose, M.; Montambault, M.; Chang, W.; Marliss, E. B. Immunological responses to chronic heat exposure and food restriction in rats. Am. J. Clin. Nutr. 48:361–367; 1988.
14. De Boer, S. F.; Koopmans, S. J.; Slangen, J. L.; Van Der Gugten, J. Plasma catecholamine, corticosterone and glucose responses to repeated stress in rats: Effect of interstressor interval length. Physiol. Behav. 47:1117–1124; 1990.
15. Deschaux, P. Neuro-immunologie: Le système immunitaire est-il un organe sensoriel? Arch. Int. Physiol. Biochem. 96:A78–A89; 1988.
16. Devoino, L.; Morozova, N.; Cheido, M. Participation of serotoninergic system in neuroimmunomodulation: Intraimmune mechanisms and the pathways providing an inhibitory effect. Intern. J. Neurosci. 40:111–128; 1988.
17. Fantino, M.; Faion, F. Lowering body weight set-point by dextrofenfluramine. In: Vague, J.; Björntorp, P.; Guy-Grand, B.; Rebuffé-Scrive, M.; Vague, P., eds. Metabolic complications of human obesities. vol. 682. International congress series. Amsterdam: Excerpta Medica; 1985:185–197.
18. Fantino, M.; Faion, F.; Rolland, Y. Effect of dexfenfluramine on body weight set-point: Study in the rat with hoarding behaviour. Appetite 7(suppl.):115–126; 1986.
19. Fillion, M. P.; Prudhomme, N.; Haour, F.; Fillion, G.; Bonnet, M.; Lespinats, G.; Masek, K.; Flegel, M.; Corvaia, N.; Launay, J. M. Hypothetical role of the serotoninergic system in neuroimmunomodulation: Preliminary molecular studies. In: Hadden, J. W.; Masek, K.; Nistico, G., eds. Interactions among CNS, neuroendocrine and immune systems. Roma: Pythagora Press; 1989: 235–250.
20. Hellstrand, K.; Hermodsson, S. Role of serotonin in the regulation of human natural killer cell cytotoxicity. J. Immunol. 139: 869–875; 1987.
21. Hillhouse, E. W.; Milton, N. G. N. Effect of acetylcholine and 5-HT on the secretion of corticotrophin-releasing factor-41 and arginine vasopressin from the rat hypothalamus in vitro. J. Endocrinol. 122:713–718; 1989.
22. Holmes, M. C.; Di Renzo, G.; Beckord, U.; Gillham, B.; Jones, M. T. Role of serotonin in the control of secretion of corticotrophin releasing factor. J. Endocrinol. 93:151–160; 1982.
23. Irwin, M. R.; Segal, D. S.; Hauger, R. L.; Smith, T. L. Individual behavioral and neuroendocrine differences in responsiveness to audiogenic stress. Pharmacol. Biochem. Behav. 32:913–917; 1989.
24. Jackson, J. C.; Cross, R. J.; Walker, R. F.; Markesbery, W. R.; Brooks, W. H.; Roszman, T. L. Influence of serotonin on the immune response. Immunology 54:505–512; 1985.
25. Kant, G. J.; Eggleston, T.; Landman-Roberts, L.; Kenion, C. C.; Driver, G. C.; Meyerhoff, J. L. Habituation to repeated stress is stressor specific. Pharmacol. Biochem. Behav. 22:631–634; 1985.
26. Kennett, G. A.; Dickinson, S. L.; Curzon, G. Central serotonergic responses and behavioural adaptation to repeated immobilisation: The effect of the corticosterone synthesis inhibitor metyrapone. Eur. J. Pharmacol. 119:143–152; 1985.
27. Krahn, D. D.; Gosnell, B. A.; Grace, M.; Lévine, A. S. CRF antagonist partially reverses CRF-and stress-induced effects on feeding. Brain Res. Bull. 17:285–289; 1986.
28. Laplanche, J. L.; Beaudry, P.; Launay, J. M.; Dreux, C.; Goussault, Y. Initial evidence for serotonin uptake by human peripheral blood lymphocytes. Biogen. Amines 3:193–200; 1985.

29. McElroy, J. F.; Miller, J. M.; Meyer, J. S. Fenfluramine, *p*-chloroamphetamine and *p*-fluoroamphetamine stimulation of pituitary adrenocortical activity in rat:evidence for differences in site and mechanisms of action. J. Pharmacol. Exp. Ther. 228: 593–599; 1984.
30. Morley, J. E.; Blundell, J. E. The neurobiological basis of eating disorders:some formulations. Biol. Psychiatry 23:53–78; 1988.
31. Morley, J. E.; Levine, A. S. Corticotrophin releasing factor, grooming and ingestive behavior. Life Sci. 31:1459–1464; 1982.
32. Morley, J. E.; Lévine, A. S.; Rowland, N. E. Stress induced eating. Life Sci. 32:2169–2182; 1983.
33. Natelson, B. H.; Creighton, D.; McCarty, R.; Tapp, W. N.; Pitman, D.; Ottenweller, J. E. Adrenal hormonal indices of stress in laboratory rats. Physiol. Behav. 39:117–125; 1987.
34. Natelson, B. H.; Ottenweller, J. E.; Cook, J. A.; Pitman, D.; McCarty, R.; Tapp, W. N. Effect of stressor intensity on habituation of the adrenocortical stress response. Physiol. Behav. 43:41–46; 1988.
35. Odio, M. R.; Maickel, R. P. Comparative biochemical responses of rats to different stressful stimuli. Physiol. Behav. 34:595–599; 1985.
36. Petrovic, L. M.; Lorens, S. A.; George, M.; Cabrera, T.; Gordon, B. H.; Handa, R. J.; Campbell, D. B.; Clancy, J., Jr. Subchronic D-fenfluramine treatment enhances the immunological competence of old female Fischer 344 rats. In: Fozard, J. R.; Saxena, P. R., eds. Serotonin: Molecular biology, receptors and functional effects. Basel: Birkhauser Verlag; 1991:389–397.
37. Pitman, D. L.; Ottenweller, J. E.; Natelson, B. H. Plasma corticosterone levels during repeated presentation of two intensities of restraint stress: Chronic stress and habituation. Physiol. Behav. 43:47–57; 1988.
38. Richardson, J.; Keeling, M. The mechanisms of stress and immunosuppression. Med. Hypotheses 7:765–780; 1981.
39. Roszman, T. L.; Jackson, J. C.; Cross, R. J.; Titus, M. J.; Markesbery, W. R.; Brooks, W. H. Neuroanatomic and neurotransmitter influences on immune function. J. Immunol. 135: 769–772; 1985.
40. Rothwell, N. J. Central effects of CRF on metabolism and energy balance. Neurosci. Biobehav. Rev. 14:263–271; 1990.
41. Rowland, N. E.; Antelman, S. M.; Kocan, D. Differences among serotonergic' anorectics in a cross-tolerance paradigm: Do they all act on serotonin systems? Eur. J. Pharmacol. 81:57–66; 1982.
42. Rowland, N. E.; Caputo, F. A.; Fregly, M. J. Water intake induced in rats by serotonin and 5-hydroxytryptophan: Different mechanisms? Brain Res. Bull. 18:501–508; 1987.
43. Rowland, N. E.; Carlton, J. Neurobiology of an anorectic drug fenfluramine. Prog. Neurobiol. 27:13–62; 1986.
44. Schettini, G.; Quattrone, A.; Di Renzo, G.; Preziosi, F. Effect of selective degeneration of brain serotonin-containing neurons on plasma corticosterone levels: Studies with D-fenfluramine. Pharmacol. Res. Comm. 11:545–553; 1979.
45. Serri, O.; Rasio, E. Temporal changes in prolactin and corticosterone response during chronic treatment with D-fenfluramine. Horm. Res. 31:180–183; 1989.
46. Shimuzi, N.; Oomura, Y.; Kai, Y. Stress-induced anorexia in rats mediated by serotonergic mechanisms in the hypothalamus. Physiol. Behav. 46:835–841; 1989.
47. Souquet, A. M.; Rowland, N. E. Effect of chronic administration of dexfenfluramine on stress and palatability-induced food intake in rats. Physiol. Behav. 46:145–149; 1989.
48. Spinedi, E.; Negro-vilar, A. Serotonin and adrenocorticotropin release: Direct effects at the anterior pituitary level and potentiation of arginine vasopressin-induced ACTH release. Endocrinology 112:1217–1223; 1983.
49. Stein, M.; Schleifer, S. J. Frontiers of stress research: Stress and immunity. In: Zales, M. R., ed. Stress in health and disease. New York: Brunner/Mazel; 1985:97–114.