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Ontogeny of Fenfluramine and Amphetamine Anorexia Compared in Rat Pups

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LESHEM, M. *Ontogeny of fenfluramine and amphetamine anorexia compared in rat pups.* PHARMAC. BIOCHEM. BEHAV. **15**(6) 859–863, 1981.—The anorexic effects of three doses of amphetamine or fenfluramine were compared in rat pups 5, 10, 15, 20 and 25 days of age. The anorexic effects of the drugs were monitored by weighing the pups before and after a 90 minute feeding period following four hours of deprivation. Amphetamine appeared to induce weight gain in 5-day-old rats, but at 10 days and thereafter, became progressively more potent in reducing weight gain and caused weight loss indicative of the involvement of non-specific factors. In contrast, while fenfluramine also reduced weight gain at 10 days, its potency was reduced in older pups and it did not cause weight loss. These results suggest that in rat pups amphetamine and fenfluramine act differently to reduce feeding and that brain serotonergic systems mediating inhibition of suckling are functional in 10-day-old rats. It is also argued that these findings lend credence to the notion that the ontogeny of feeding parallels its recovery after brain lesions.

Amphetamine Anorexia Feeding Fenfluramine Rat pups

THE anorexic influence of fenfluramine and amphetamine in neonates is of interest on several counts.

First, although it has been shown that amphetamine anorexia emerges after 15 days of age [10], in adult rats the anorexic effects of amphetamine and fenfluramine have been repeatedly dissociated [3, 4, 6, 7] and it seems unlikely that amphetamine's influence in neonates can be predictive of the action of fenfluramine or other anorexic drugs.

Second, it appears from the data presented in the amphetamine study [10] that higher doses than those tested may, in fact, be anorexic sooner than 15 days. Moreover, since drug induced weight loss (as distinct from prevention of weight gain) was apparent in that study, and such weight loss must be due to factors other than reduced feeding, it is important to consider the relative contribution of non specific effects to weight loss.

Third, it has been suggested that changes with age in the action of pharmacological agents on feeding in neonatal rats might reflect on the ontogeny of the neurochemical substrates of feeding [10, 11, 14]. While there is considerable data on the ontogeny of monoamine systems in developing rat brain (e.g. [1,9]) there are few clues as to their functional maturity in terms of the emergent behavioural repertoire these systems may subserve. However, it has been shown recently that 5-HT agonists and antagonists influence suckling behaviour after 15 days age in a pattern consistent with an inhibitory role for brain 5-HT in the inhibition of suckling [11,14]. Fenfluramine anorexia is believed to depend on a central serotonergic system [6, 7, 12] so that the efficacy of fenfluramine in the neonate could provide further indication of the functional status of the serotonergic system with respect to feeding.

Finally, it has been proposed that the ontogeny of feeding may parallel its recovery after brain damage [11,13]. Since fenfluramine and amphetamine are differently influenced by brain lesions and these effects follow distinct courses of recovery [4], a comparison of the drugs' effects in neonates could be pertinent to this proposal.

The present study addresses these issues by comparing the anorexic potencies of amphetamine and fenfluramine in rat pups of various ages.

METHOD

Subjects

90 litters of Charles Rivers city strain derived rat pups and their dams were tested. The pups were born in the lab and litter size was reduced to 6 on the first day *post-partum*.

Each litter with its dam was maintained in a wire cage with an aluminum nesting box. Shredded paper was supplied for nesting. Lights were on from 0700 to 1900 hr and the temperature maintained around 21°C. throughout the experiment.

Design

Body weight changes were recorded after a 90 minute suckling session which followed 4 hours of deprivation.

There were eight treatment conditions: amphetamine and fenfluramine at 3 dose levels each, a "Saline" control and a "No Feeding" control. All treatment conditions were tested at each of 5 ages: 5, 10, 15, 20 and 25 days *post-partum*. Each pup was tested only once.

Procedure

Dams and food hoppers were removed at 1200 hr. At 1530

TABLE 1

SIGNIFICANT DIFFERENCES BETWEEN DRUG AND CONTROL TREATMENTS

Drug	Dose (mg/kg)	Compared to Saline: Age in days 5	10	15	20	25	Compared to "No Feeding": Age in days 5	10	15	20	25
					a					b	
	1.0				05*	01		01		01	05*
Amphetamine	3.0			01†	01	01	01†	01		05*	**01**
	9.0	‡	01	01	01	01	01		**01**		**01**
					c				d		
	0.5			01	05*	01				01	01
Fenfluramine	1.5		01	01	01	01			05	01	01
	4.5		01	01	05	01				01	01

Values in the body of the Table are minimal decimal probabilities for significant differences from control treatments in Figs. 1 and 2. Empty cells, $p<0.05$. (*) proportional measure only (†) difference measure only. The bold numbers in Table 1b denote weight loss greater than the "No Feeding" condition. (‡) See Fig. 2.

hr the pups were individually marked with picric acid, stroked in the anogenital region to induce urination, weighed to within 0.1 g and injected with drugs 20 min before the dams were returned to the cages. Dams were returned at 1600 hr and the pups allowed to feed for 90 min. The pups were then reweighed.

Treatments

dl-Amphetamine sulphate (1.0, 3.0 and 9.0 mg/kg) and dl-fenfluramine hydrochloride (Abic, Ltd, Israel) (0.5, 1.5 and 4.5 mg/kg) in saline vehicle were injected in volumes of 0.01 ml/gm body weight. The injections were administered abdominally using a 27 gauge needle. Saline served for control injections.

In the "No Feeding" condition, litters were raised with their dam and a preparous female introduced prenatally. At deprivation both adults were removed but only the nonlactating female was returned for the 90 minute test interval. This condition was intended to establish the weight loss due to total inhibition of feeding *per se* so as to provide an indication of the contribution of anorexia while excluding other drug effects causing weight loss. This control was instituted since a previous study has shown absolute weight loss after drug treatment in pups [10] which cannot be due to suppression of suckling alone.

The saline and "No Feeding" controls thus set limits between which changes in body weight can reasonably be attributed to drug induced anorexia.

RESULTS

Two parallel ANOVA's were performed: one on the absolute weight change (i.e., the difference between body weight before and after feeding), and the other on a proportional measure (i.e., the difference in weight divided by body weight before the test). Statistics are given for the difference measure, and the significances did not differ between the two measures except where stated. Significant differences were established with Dunnet's t statistic.

Controls

Saline treatment showed a main effect attributable to age, $F(4,59)=36.3$, $p<0.001$, indicating that pups gain more weight during 90 min of feeding as they grow older (Figs. 1 and 2). The "No Feeding" condition caused a weight loss which was more pronounced with age, $F(4,54)=37.3$, $p<0.001$ (Figs. 1 and 2). Weight loss relative to saline was evident at all ages tested ($p<0.02$ at 5 days, p's<0.001 all other ages, Figs. 1 and 2). A powerful two-way interaction of treatments with age, $F(4,113)=62$, $p<0.001$, confirmed that the progressive effects of age on body weight change in the two treatments are different.

Anorexic Drugs

Amphetamine showed a two-way interaction of dosage and age, $F(8,186)=10.6$, $p<0.001$, indicating that the potency of amphetamine dosage changes with age. Inspection of Fig. 1 suggests that this effect is one of a progressive increase in body weight loss with the greater increase occurring with higher dosage. The progressive nature of the increase in amphetamine anorexia with age and dose is clearly seen in Table 1a.

In contrast, no such interaction was obtained for fenfluramine. Figure 2 shows that the relative potency of fenfluramine doses was stable with age.

Amphetamine treatment overall did not differ from the "No Feeding" condition, $F(1,250)=0.61$, $p=0.44$. Inspection of Fig. 1 reveals that the lack of overall effect was probably due to respectively lesser and greater weight loss relative to "No Feeding" with age.

Again, fenfluramine differed in showing a treatment effect with "No Feeding," $F(1,232)=31.2$, $p<0.001$, and this is probably because of changes after 15 days of age (Fig. 2).

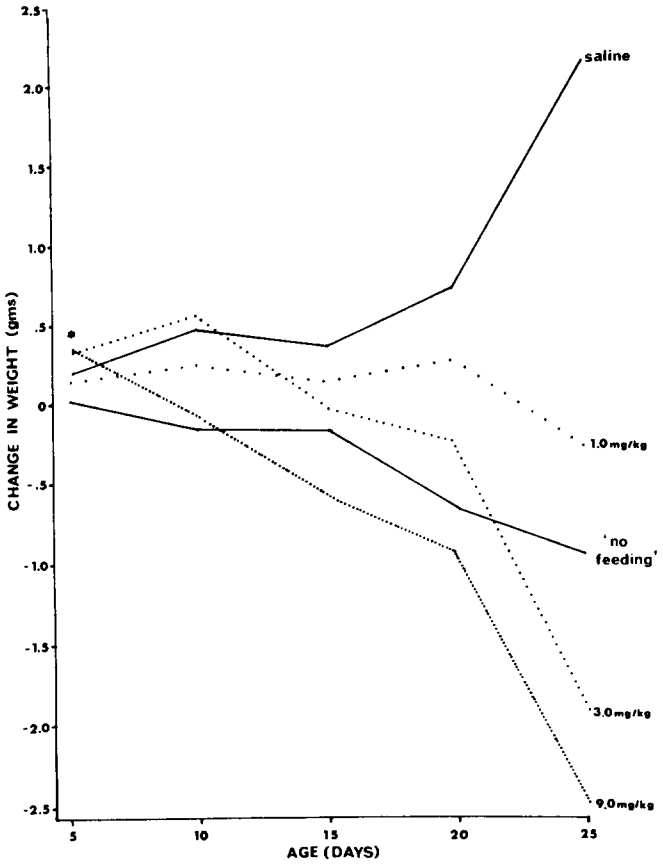

FIG. 1. Influence of control treatments and amphetamine by age and dose on body weight during the 90 minute suckling test. *9.0 mg/kg different from saline, $p<0.05$ (proportional measure). See Table 1 for other significant differences.

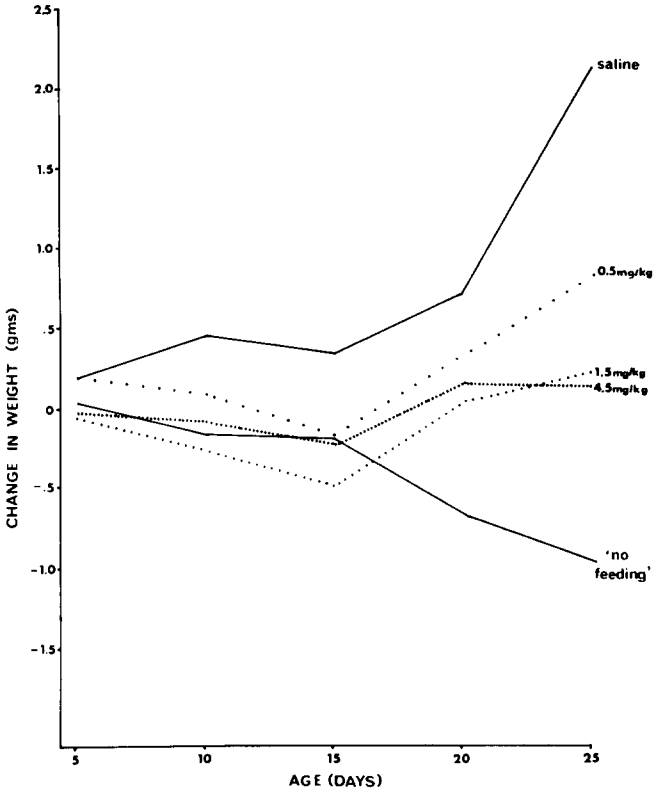

FIG. 2. Influence of control treatments and fenfluramine by age and dose on body weight during the 90 minute suckling test. See Table 1 for significant differences.

Thus fenfluramine becomes *less* potent than "No Feeding" after 15 days of age, failing to maintain its previous capacity to totally inhibit feeding (Table 1d) and effecting only an approximate 50% reduction compared to Saline, $F(1,237)=67.8$, $p<0.001$.

Fenfluramine anorexia was apparent at 5 days (Fig. 2) but just failed to attain significance probably because the difference between Saline and "No Feeding" was itself mild at this age (0.16 ± 0.06 g, $p<0.02$).

The total inhibition of feeding before 15 days explains the absence of dose-dependence suggesting a "floor effect" with even 0.5 mg/kg fenfluramine. Moreover, since amphetamine also caused maximal anorexia, the different effects of the two drugs cannot be ascribed to lack of dose equivalence.

These differences were reflected in ANOVA for drug effects revealing that the drugs differed in their influence on body weight, $F(1,354)=26.9$, $p<0.001$. There was a powerful two-way interaction of treatment and age, $F(4,354)=54.0$, $p<0.001$. This dissociation between the courses of amphetamine and fenfluramine anorexia over age was supported by one-way trend analysis showing that amphetamine's effect with age deviated from a cubic, $F(1,196)=4.5$, $p<0.03$, and lower order expressions, while fenfluramine's effects matched a quadratic expression, $F(2,178)=2.5$, $p>0.08$.

DISCUSSION

The experiments show that the ontogeny of fenfluramine and amphetamine anorexia differs in neonate rats. Fenfluramine anorexia is manifest at 10 days of age and its efficacy decreases between 15 and 25 days of age. In contrast, amphetamine increases intake at 5 days and thereafter there is an increasing anorexic effect with age and dose. Moreover, amphetamine causes weight loss in pups over and above that attributable to anorexia. There is, therefore, a marked divergence of the response curves of the two drugs after 15 days of age.

Amphetamine's hyperphagic effect at 5 days has apparently not been reported previously. However, this finding is not at variance with the suggestion that amphetamine-increased nipple attachment is a consequence of heightened arousal [2] nor does it conflict with the data in graphs presented in a study of amphetamine in 5-day-old pups although the authors do not comment on that data [10].

The onset of amphetamine anorexia is less precisely demarcated than the previously suggested 15 days and is dose related: 9.0 mg/kg amphetamine causes weight loss at 10 days of age and lower doses take effect in age related fashion with 1.0 mg/kg effecting anorexia only after 20 days of age. At face value this could suggest a progressive susceptibility of the catecholamine substrates of amphetamine anorexia starting after 5 days. Nevertheless, such an inference should be viewed with caution in the face of the evident involvement of factors additional to inhibition of feeding in weight

loss caused by amphetamine injection, since weight loss in older pups was greater than in the "No Feeding" condition. Moreover, there may be an amphetamine-sensitive substrate that is nevertheless functionally immature, since different components of the catecholamine synaptic mechanism may mature at different rates [5].

Fenfluramine's suppression of suckling is in line with its 5-HT agonist properties [6, 7, 12] and a proposed inhibitory role for 5-HT in suckling [11,14].

According to this proposal, 5-HT receptors are present at birth, but presynaptic 5-HT mechanisms mature some 15 days later. Thus, while 5-HT agonists acting on receptors inhibit suckling even before 15 days-of-age, receptor blockers will disinhibit suckling only after that age when endogenous release of 5-HT commences.

This scheme can account for the present findings. Fenfluramine inhibits suckling as early as 10 days *post-partum* presumably by a direct receptor action [12] and/or by releasing and inhibiting reuptake of available 5-HT [6,7], although the latter proposition does place constraints on the possible nature of the immature presynaptic components. Fenfluramine's reduced potency after 15 days (see also the comparatively mild effect of 10 mg/kg [14]) can be explained by competition with endogenously released 5-HT.

One central aspect of Williams *et al.'s* [14] proposals remains unclarified. Serotonergic suppression of suckling, as evidenced by their data and the present findings, can be of two types: (1) a tonic suppression commencing 15 days *post-partum* and leading to weaning, (2) an acute suppression mediating factors regulating episodes of suckling. These functions are not necessarily exclusive but they do warrant considered investigation.

Finally, while it is always difficult to ascertain the specificity of drug action, it is noteworthy that, in contrast to amphetamine, fenfluramine does not cause weight loss even at 3 times the dose required to effect total inhibition of weight gain due to suckling. It seems clear therefore, that fenfluramine's influence on weight change is less compounded by non-specific effects.

At this juncture it is perhaps illuminating to consider two views of early postnatal feeding behaviour. In an admirable analysis, Blass, Hall and Teicher [2] characterize the regulation of intake before approximately 2 weeks of age as subject to the indirect control of external contingencies. In contrast, Houpt and Epstein [8] note the role of upper gastrointestinal tract repletion in the control of feeding in newborn pups.

These distinct views might well reflect the respective experimental emphases of these authors on hunger and satiety, and it can be argued that the effects of amphetamine are consistent with Blass *et al.*'s [2] views, and those of fenfluramine with Houpt and Epstein's [8]. This argument rests on the characterization of amphetamine anorexia as consequent on hunger reduction, that of fenfluramine on its satiety inducing effect [3]. Consistent with the present findings, therefore, amphetamine can be expected to exert no anorexia until the central controls of appetitive behaviour mature as proposed by Blass *et al.* [2]. Indeed, as these authors suggest, increased arousal induced by amphetamine could cause hyperphagia where external stimuli are the dominant instigators of feeding. In this vein, the early onset of fenfluramine anorexia in terms of increased satiety is compatable with the existence of post consummatory inhibitory mechanisms, as proposed by Houpt and Epstein [8], possibly reducing the saliency of sensory cues [14].

Finally, the ontogeny of amphetamine and fenfluramine anorexia lends powerful support to the notion of the recapitulation of the ontogeny of feeding in its recovery [13]. After lateral hypothalamic lesions, fenfluramine anorexia is enhanced, that of amphetamine attenuated. Moreover, there is a subsequent recovery during which these effects are gradually reversed [4]. The parallels with increasing potency of amphetamine and apparently reduced potency of fenfluramine in neonates are remarkable. Equally striking is the finding that 5 days after birth amphetamine causes hyperphagia, as it does shortly after lateral hypothalamic lesions [15]. These findings, therefore, expand the list of similarities in drug effects in the ontogeny and recovery of feeding compiled hitherto [8, 10, 11, 13].

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REFERENCES

1. Asano, Y. The maturation of the circadian rhythm of brain norepinephrine and serotonin in the rat. *Life Sci.* **10:** 883–894, 1971.
2. Blass, E. M., W. G. Hall and M. H. Teicher. The ontogeny of suckling and ingestive behaviours. In: *Progress in Psychobiology and Physiological Psychology,* Vol. 8, edited by J. M. Sprague and A. M. Epstein. New York: Academic Press, 1979, pp. 243–299.
3. Blundell, J. E., C. J. Latham and M. Leshem. Differences between the anorexic actions of amphetamine and fenfluramine—possible effects on hunger and satiety. *J. Pharm. Pharmac.* **28:** 471–477, 1976.
4. Blundell, J. E. and M. Leshem. Central action of anorexic agents: effects of amphetamine and fenfluramine in rats with lateral hypothalamic lesions. *Eur. J. Pharmac.* **28:** 81–88, 1974.
5. Coyle, J. T. and M. E. Molliver. Major innervation of newborn rat cortex by monoaminergic neurons. *Science* **196:** 444–447, 1977.
6. Duhault, J., L. Beregi, P. Gonnard and M. Boulanger. Brain serotonergic system and anorectic drugs. In: *Central Mechanisms of Anorectic Drugs,* edited by S. Garattini and R. Samanin. New York: Raven Press, 1978, pp. 205–215.
7. Garattini, S., E. Borroni, T. Menini and R. Samanin. Differences and similarities among anorexic agents. In: *Central Mechanisms of Anorectic Drugs,* edited by S. Garattini and R. Samanin. New York: Raven Press, 1978, pp. 127–143.
8. Houpt, K. A. and A. N. Epstein. Ontogeny of controls of food intake in the rat. GI fill and glucoprivation. *Am. J. Physiol.* **225:** 58–66, 1973.
9. Loizou, L. A. The postnatal ontogeny of monoamine containing neurons in the central nervous system of the albino rat. *Brain Res.* **40:** 395–418, 1972.
10. Lytle, L. D., W. H. Moorcroft and B. A. Campbell. Ontogeny of amphetamine anorexia and insulin hyperphagia in the rat. *J. comp. physiol. Psychol.* 77: 388–393, 1971.

11. Nock, B., C. L. Williams and W. G. Hall. Suckling behavior of the infant rat: Modulation by a developing neurotransmitter system. *Pharmac. Biochem. Behav.* **8:** 277–280, 1978.
12. Sugrue, M. F., I. Goodlet and I. McIndewar. Failure of depletion of rat brain 5-HT to alter fenfluramine-induced anorexia. *J. Pharm. Pharmac.* **27:** 950–953, 1975.
13. Teitelbaum, P., M. Cheng and P. Rozin. Development of feeding parallels its recovery after hypothalamic damage. *J. comp. physiol. Psychol.* **67:** 430–441, 1969.
14. Williams, C. L., J. S. Rosenblatt and W. G. Hall. Inhibition of suckling in weaning age rats: a possible serotonergic mechanism. *J. comp. physiol. Psychol.* **93:** 414–429, 1979.
15. Wolgin, D. L., J. Cytawa and P. Teitelbaum. The role of activation in the regulation of food intake. In: *Hunger: Basic Mechanisms and Clinical Implications*, edited by D. Novin, W. Wyrwicka and G. Bray. New York: Raven Press, 1976, pp. 179–191.
16. Zigmond, E. M. and M. J. Stricker. Recovery of function after damage to central catecholamine containing neurons: a neurochemical model for the lateral hypothalamic syndrome. In: *Progress in Psychobiology and Physiological Psychology*, Vol. 9, edited by J. M. Sprague and A. E. Epstein. New York: Academic Press, 1976, pp. 121–188.