Pharmacology Biochemistry & Behavior, Vol 24 pp 299–304 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

The Effect of Diet and Chronic Obesity on Brain Catecholamine Turnover in the Rat

BARRY E LEVIN,*[1] JOSEPH TRISCARI† AND ANN C. SULLIVAN†

**Neurology Service, Veterans Administration Medical Center, E Orange, NJ 07019*
Department of Neurosciences, New Jersey Medical School, Newark, NJ 07103
and †Department of Pharmacology, Hoffmann-La Roche, Nutley, NJ 07110

Received 16 January 1985

LEVIN, B E , J TRISCARI AND A C SULLIVAN *The effect of diet and chronic obesity on brain catecholamine turnover in the rat* PHARMACOL BIOCHEM BEHAV 24(2) 299–304, 1986 —Male Sprague-Dawley rats were fed a high calorie, high fat diet for 3 months to produce chronic diet-induced obesity (DIO) in which they gained 70% more weight than chow-fed controls Thirty-six percent of the rats fed the DIO diet resisted the developmentof obesity (DR), gaining no more weight than chow-fed controls but serving as a comparison for the effects of the diet alone on the metabolism of brain catecholamines The major influence of dietary composition was upon norepinephrine (NE) metabolism Both DIO and DR rats had increased turnover of NE (107–217%) and/or shorter NE half-lives (42–67%) than controls in the hypothalamic paraventricular (PVN) and dorsomedial (DMN) nuclei and the median eminence (ME), dopamine (DA) turnover was similarly accelerated in the PVN The DR rats alone exhibited decreased NE levels with increased disappearance of NE in frontal cortex and increased disappearance of DA in the ventromedial hypothalamic nucleus (VMH) The major effect of chronic obesity alone was a 31–33% decrease in DMN DA turnover and an 80% decrement in ME DA turnover associated with a 61% decrease in DA levels as compared to chow-fed controls Therefore, the major effect of a high calorie, high fat diet was a diffuse acceleration of brain NE and DA turnover while chronic obesity led to decreased DA turnover in the DMN and ME

Diet-induced obesity Norepinephrine Dopamine Epinephrine Catecholamines Hypothalamus Frontal cortex

A number of studies have shown that changes in diet may be associated with altered metabolism of brain catecholamines [1, 7, 32] In addition, states of chronic obesity in rodents, whether genetically determined [5, 6, 16, 17] or lesion-induced [4], have also been linked to changes in brain monoamine levels Diet-induced obesity (DIO) can be produced in rats by feeding them high calorie, high fat diets over a period of weeks to months [8, 15, 19, 20, 30] This method of causing obesity obviates many of the problems associated with genetically-linked or lesion-induced types of animal obesity in which many other defects which are not necessarily causally related to the pathophysiology of obesity are also present As such, DIO has many similarities to certain types of adult-onset obesity in humans These include hypercellular, hypertrophic adiposity in some fat depots [8,36], type II diabetes with insulin resistance [7, 18, 36], hyperglycerolemia, and an associated increase in metabolic efficiency such that increased body weight is maintained with the intake of the same or fewer calories than comparable lean controls [15, 18, 21, 36]

We have previously used a semisynthetic diet which is relatively high in calories and fat ("DIO diet") to produce DIO in adult rats [15, 19, 20, 21] Generally, rats show an early increase in food intake on this diet with the subsequent development of obesity Over the ensuing 2–3 month period caloric intake is usually reduced to levels fairly comparable to or even below that of chow-fed controls [15, 20, 21, 36] In some of these chronic studies, a subgroup of rats fed the DIO diet fails to increase its initial intake and thus "resists" the development of DIO [15,20] These diet-resistant (DR) rats thus serve as a useful control for the effects of dietary composition on the parameters to be examined The presence of such a group in a study alleviates the problems associated with restricted feeding schedules which would otherwise be required to produce a dietary control group of comparable weight to chow-fed controls Thus the use of our DIO diet provides an extremely powerful animal model which can serve as a means to evaluate the effects of both high calorie, high fat diets and chronic obesity on the metabolism of brain catecholamines

METHOD

Animals

Three month old (400–500 g), adult, male Sprague-Dawley rats (Charles River Breeding Laboratories) were

[1]Requests for reprints should be addressed to Barry E Levin, Neurology Service (127), VA Medical Center, East Orange, NJ 07019

TABLE 1

BODY WEIGHT CHANGES IN RATS FED CHOW OR THE DIO DIET

	Chow	DIO	DR
Initial Body Weight (g)	417 ± 6	420 ± 6	420 ± 6
Final Body Weight (g)	655 ± 21	824 ± 15*	664 ± 13
Weight Gain (g)	238 ± 18	405 ± 13*	251 ± 10
N	30	29	16

Groups of rats were fed chow (n=30) or the DIO diet (n=45) for 3 months A subgroup of rats on this later diet (n=16) resisted the development of obesity (DR) while the remainder became obese (DIO) *$p<0.001$ when DIO rats were compared to chow-fed controls by post-hoc t-test after a significant intergroup difference was found by ANOVA

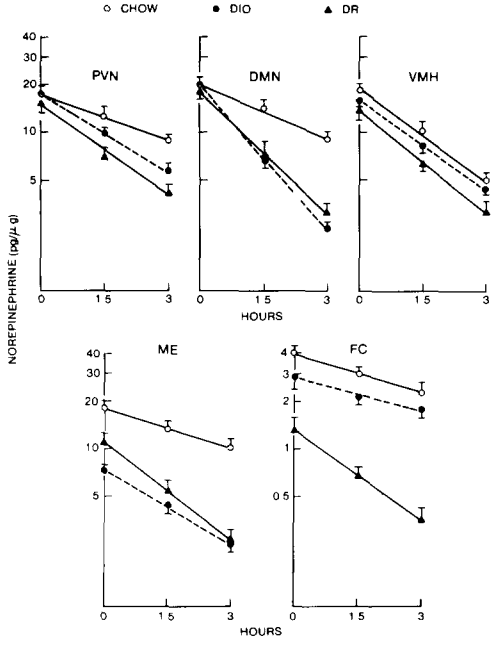

FIG 1 Effect of diet and obesity on brain NE metabolism Rats were fed chow (○) or the DIO diet Subgroups of the DIO diet-fed rats became obese (●) or resisted the development of obesity (▲) over a 3 month period of feeding Sets of 4–10 rats from each of the 3 diet groups were treated with α-methyl p-tyrosine (300 mg/kg, IP) and decapitated 1 5 and 3 hr later A further set of 8–14 rats from each diet group was left untreated for determination of endogenous NE levels Data points are mean±SE (vertical bars) NE concentration (log) plotted as a function of time for the hypothalamic paraventricular (PVN), dorsomedial (DMN), ventromedial (VMH) nuclei, median eminence (ME) and frontal cortex (FC) Turnover data derived from these plots are given in Table 2

housed individually at 23–24°C on a 12 hr light-dark schedule Rats were randomized by weight into 2 groups, controls (n=30) were fed pelleted Purina rat chow which contains 4 5% fat and 4 0 kcal/g and a second group (n=45) was fed a pelleted, semisynthetic diet (BioServ, Inc) composed of 47% chow, 8% corn oil and 44% sweetened condensed milk containing 16–17% protein, 15–16% fat and 4 6 kcal/g (DIO diet, [19]) Diet-feeding was continued for 3 months at the end of which time rats were weighed and allocated to 1 of 3 groups according to analysis of body weight-gain histograms [15, 20, 21] Chow-fed rats showed a Gaussian distribution of body weight gain while DIO diet-fed rats fell into 2 subgroups, both of which were "normally' distributed according to body weight gain The first group became obese (DIO), gaining significantly more weight than controls (Table 1), the second group ate the DIO diet but gained approximately the same amount of weight as controls and these were defined as "diet-resistant" (DR) All further studies were carried out prospectively upon these animals as previously allocated to these 3 groups

TABLE 2

EFFECT OF DIET AND OBESITY ON BRAIN NE METABOLISM

	Chow	DIO	DR
PVN			
NE_0	15 3 ± 4 90	17 3 ± 2 60	16 3 ± 0 80
$t^1/_2$	3 55 ± 0 69	1 94 ± 0 41*	1 50 ± 0 32†
TR	2 99 ± 0 77	6 18 ± 1 12*	7 62 ± 1 00†
DMN			
NE_0	18 9 ± 2 7	20 6 ± 2 5	18 7 ± 1 9
$t^1/_2$	2 85 ± 0 72	0 98 ± 0 13†	1 17 ± 0 16ˣ
TR	4 60 ± 0 91	14 6 ± 1 85‡	11 1 ± 1 32‡
VMH			
NE_0	18 3 ± 2 1	16 0 ± 1 7	14 2 ± 3 5
$t^1/_2$	1 62 ± 0 25	1 63 ± 0 21	1 39 ± 0 32
TR	7 83 ± 1 05	6 80 ± 0 80	7 08 ± 1 69
ME			
NE_0	18 6 ± 1 9	7 24 ± 0 9‡	10 8 ± 2 2ᵀ
$t^1/_2$	3 63 ± 0 87	1 98 ± 0 45*	1 48 ± 0 34*
TR	3 55 ± 0 61	2 53 ± 0 45	5 06 ± 1 10
FC			
NE_0	4 04 ± 0 53	2 89 ± 0 50	1 32 ± 0 24ᵀ§
$t^1/_2$	3 86 ± 0 87	4 56 ± 1 49	1 69 ± 0 37*
TR	0 73 ± 0 13	0 44 ± 0 11	0 55 ± 0 11

Rats fed chow or the DIO diet for 3 months (those which became obese [DIO] and those which did not [DR]) were given α-methyl p-tyrosine (300 mg/kg, IP) and decapitated in sets of 4–10 at 1 5 and 3 hr later Other untreated rats were used for endogenous NE levels (NE_0, pg/μg protein) Data were plotted as in Fig 1 and expressed as mean ± SE half-life ($t^1/_2$, hr^{-1}) and turnover rate (TR, pg/μg protein/hr) *$p<0.05$ †$p<0.01$ ‡$p<0.001$ when data from DIO or DR rats were compared to those from chow-fed rats and §$p<0.05$ when data from DIO rats were compared to those from DR rats by t test for post-hoc comparisons after ANOVA showed there to be significant intergroup differences ($p<0.05$)

Brain Catecholamine Turnover Studies

Subgroups of rats from each of the 3 diet groups were injected with α-methyl p-tyrosine (300 mg/kg, IP) and 4–10 of these rats from each grup were decapitated 1 5 hr or 3 hr later [2,12] Additional animals from each diet group (n=8–14) were not injected with drug and were used for the determination of endogenous brain catecholamine levels At the time of decapitation, brains were quickly removed, frozen on Dry Ice and stored at −70°C for no more than 2 days Brains

TABLE 3

EFFECT OF DIET AND OBESITY ON BRAIN DA METABOLISM

	Chow	DIO	DR
PVN			
DA_0	1 27 ± 0 22	2 79 ± 0 63	3 01 ± 1 34
$t^1/_2$	1 13 ± 0 24	0 97 ± 0 10	1 02 ± 0 20
TR	0 78 ± 0 15	1 99 ± 0 33*	2 05 ± 0 66*
DMN			
DA_0	3 49 ± 0 72	3 19 ± 0 57	3 49 ± 0 93
$t^1/_2$	1 32 ± 0 13	1 76 ± 0 22*	1 11 ± 0 21
TR	1 83 ± 0 15	1 26 ± 0 26*	2 18 ± 0 50
VMH			
DA_0	2 50 ± 0 48	3 39 ± 0 12	2 80 ± 0 78
$t^1/_2$	1 30 ± 0 27	1 64 ± 0 41	0 95 ± 0 20§
TR	1 33 ± 0 27	1 43 ± 0 20	2 04 ± 0 10§
ME			
DA_0	22 9 ± 3 0	8 97 ± 1 99†	25 4 ± 1 5
$t^1/_2$	0 96 ± 0 08	1 92 ± 0 40*	1 07 ± 0 14
TR	16 5 ± 1 8	3 24 ± 0 70‡	16 5 ± 1 56

Rats were fed chow or the DIO diet and treated with α-methyl p-tyrosine as described in Table 2 and the Method section DA_0=endogenous DA content (pg/μg protein) and TR=turnover rate in pg/μg protein/hr Data are derived from plots in Fig 2 and are given as mean ± SE *$p<0$ 05 †$p<0$ 01 ‡$p<0$ 001 when data from DIO or DR rats were compared to those from chow-fed rats and §$p<0$ 05 when data from DIO rats were compared to those from DR rats by t-test for post-hoc comparisons after significant intergroup differences ($p<0$ 05) were found by ANOVA

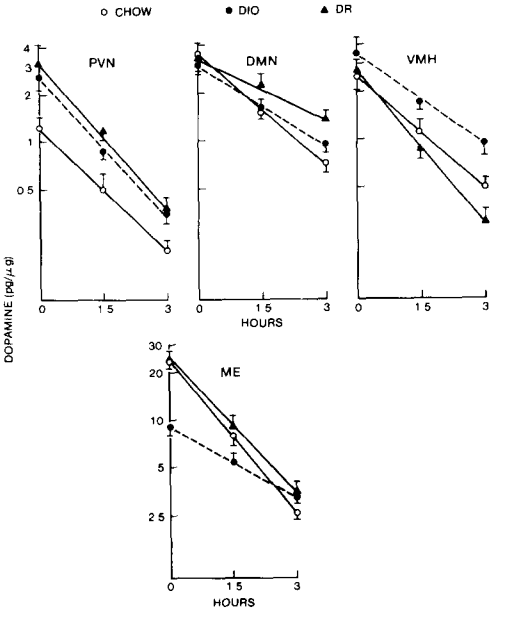

FIG 2 Effect of diet and obesity on brain DA metabolism See legend of Fig 1 and the Method section for details Data derived from these plots are given in Table 3

were microdissected by the method of Palkovits *et al* [28]. Four-6 bilateral punches were taken using a 0 5 mm punch for the hypothalamic paraventricular (PVN), dorsomedial (DMN), ventromedial nuclei (VMN) and median eminence (ME) and 4 punches from frontal cortex (FC) were taken using a 1 mm punch The tissue punches were placed immediately into 150 μl 0 1 N perchloric acid containing 10 ng/mg dihydroxybenzylamine as an internal standard Catecholamines were adsorbed to alumina at pH 8 5 and eluted with 0 1 N perchloric acid Brain catecholamines were determined by reversed phase high performance liquid chromatography using a C_{18}, μ Bondapack, 10 μm Z-module column (Waters) at a flow rate of 1 5 ml/min Electrochemical detection was performed with a glassy-carbon electrode (BAS) at an applied voltage of 0 72 v [14] Proteins were determined by the method of Lowry *et al* [22]

Statistics

Catecholamine turnover was calculated by the method of Brodie *et al* [2] in which the fractional turnover (k) was taken as the slope^{-1} of the disappearance of catecholamine levels (as a natural logarithmic function) plotted against the time following α-methyl p-tyrosine injections Half-life ($t^1/_2$) was calculated as 0 693/k Endogenous norepinephrine (NE_0), dopamine (DA_0) and epinephrine (E_0) levels were obtained from subgroups of uninjected rats and turnover rate was given as the product of endogenous levels × fractional turnover (k) Data were analyzed by 1-way analysis of variance for individual parameters and by analysis of covariance for turnover data Where significant differences ($p<0$ 05 or less) were found, data were further analyzed by t-test for post-hoc comparisons

RESULTS

Body Weight Gain

Rats which became obese after 3 months on the DIO diet had final body weights which were 24% and 26% higher than chow-fed controls and DR rats respectively (Table 1), $F(2,42)=32$ 16, $p=0$ 0001 This represented a 70% and 61% greater weight gain than these 2 groups, respectively, $F(2,42)=43$ 78, $p=0$ 0001

Brain Catecholamine Turnover

Norepinephrine Endogenous NE (NE_0) levels were decreased by 42% and 61% in the median eminence (ME) of DR and DIO rats, respectively, compared to chow-fed controls (Table 2), $F(2,21)=14$ 73, $p=0$ 001 NE_0 levels were also decreased in frontal cortex (FC) of DR rats by 67% and 54% compared to controls and DIO rats, respectively, $F(2,22)=6$ 45, $p=0$ 007 The fractional turnover of NE was increased in the PVN, DMN and the ME of both DIO and DR rats Thus the $t^1/_2$ was reduced to 55% and 34% of control and the turnover rate was increased to 207% and 255% in the PVN of DIO and DR compared to chow-fed controls, respectively (Table 2 and Fig 1), $F(2,30)=73$ 06, $p=0$ 0001 Decreases in $t^1/_2$ to 34% and 41% of control and increases in turnover rate to 317% and 241% of controls were seen in the

TABLE 4
EFFECT OF DIET AND OBESITY ON BRAIN E LEVELS

	Chow	DIO	DR
PVN	2 54 ± 0 45	2 60 ± 0 54	1 45 ± 0 50
DMN*	2 34 ± 0 26	1 81 ± 0 44	1 86 ± 0 42
VMH	1 27 ± 0 20	1 60 ± 0 19	1 92 ± 0 46
ME	1 69 ± 0 34	1 22 ± 0 36	1 67 ± 0 40

Rats fed as described in Table 2 and the Method section had endogenous E levels determined in sets of 8–14 per diet group Data are mean ± SE pg/μg protein *$F(2,19)=19\ 52$, $p=0\ 001$ for 1 way ANOVA among data for chow-fed, DIO and DR rats, there were no significant differences among the groups by t-test for post-hoc comparisons

DMN of DIO and DR rats, respectively, $F(2,30)=88\ 40$, $p=0\ 0001$ The half-life of NE was also decreased to 55% and 41% of controls in the ME of DIO and DR rats, respectively, $F(2,30)=58\ 18$, $p=0\ 0001$, but because of the diminished NE_0 levels, there were no differences in overall turnover rate In FC there was also a 57% decrease in NE $t^1/_2$ in DR rats without a concomitant increase in turnover rate, $F(2,30)=40\ 43$, $p=0\ 0001$, no change was seen in FC of DIO rats There were no differences in NE metabolism among the 3 groups in the VMH

Dopamine In contrast to the general trend toward increased turnover of NE in many of the areas sampled in both DIO and DR rats, DA metabolism was differentially affected in the DIO rats when compared to both control and DR rats (Table 3 and Fig 2) Endogenous levels of DA were decreased to 39% of chow-fed controls, $F(2,22)=5\ 92$, $p=0\ 009$, with a 100% increase in $t^1/_2$ and 80% decrease in DA turnover rate in the ME of DIO rats when compared to controls, $F(2,30)=69\ 12$, $p=0\ 0001$ There was no comparable change in DA metabolism in the ME of DR rats DIO rats also had a prolonged $t^1/_2$ for DA of 33% and a decrement in turnover rate of 31% in their DMN as compared to chow-fed controls, $F(2,30)=20\ 20$, $p=0\ 0001$ Again, DR rats showed no comparable changes in DMN DA metabolism While neither the DIO nor DR rats had significantly different DA metabolism from controls in their VMH, when they were compared to each other the DIO rats did have a 70% slower rate of VMH DA turnover as compared to the DR rats, $F(2,30)=22\ 53$, $p=0\ 0001$ Finally, while there were significant differences in neither DA_0 nor DA $t^1/_2$ in the PVN of DIO and DR rats as compared to controls, the turnover rates were increased by 155% and 163% respectively, $F(2,30)=23\ 22$, $p=0\ 0001$

Epinephrine There was no significant turnover of E following the administration of α-methyl p-tyrosine in any brain area examined Endogenous E levels were similar in all brain areas except the DMN where there were significant intergroup differences by ANOVA, $F(2,19)=19\ 52$, $p=0\ 0001$, but none by post hoc t test comparisons

DISCUSSION

Both diet and chronic obesity appear to exert independent effects upon brain catecholamine metabolism In the present studies, chronic intake of a diet which was relatively high in fat and calories, whether or not the animals became obese, had its primary effects on brain NE metabolism These changes included increased turnover in the PVN and DMN of both DR and DIO rats as well as less striking dietary effects upon DA metabolism in the PVN In addition, only the DR rats showed the combination of decreased NE levels and increased fractional turnover of NE in the ME and frontal cortex These latter changes suggest a situation in which neuronal activity and transmitter release and/or degradation of NE exceeded the synthetic capacity of the cells in producing NE In contrast to the predominantly facilitatory effect of diet on brain NE metabolism, chronic obesity alone was associated primarily with decreased DA turnover in the DMN and ME Such differential effects of diet and obesity suggest that these central sites may play an important role in the development and maintenance of chronic DIO

One possible cause for the differences in brain catecholamine metabolism seen among the groups in the present study would be possible differences in the amount of food or calories taken in Initially, those rats which are destined to become obese on the DIO diet generally increase their total caloric intake while those which will eventually become obesity-resistant tend to eat less from their earliest exposure to the diet than either chow-fed controls or DIO rats [15] After 3 months, caloric intake in DIO rats is usually fairly comparable to, or even decreased below that of chow-fed controls [15,21] Therefore, even though food intake was not measured in the current studies, it is unlikely that the differences in caloric intake alone were the determining factor in producing the changes in brain NE metabolism seen here

Neither was there a clear relationship between the amount of dietary protein intake and the changes in brain catecholamine metabolism A direct correlation between dietary protein content and ME levels of DA has been reported [9] However, while the amount of protein in chow and the DIO diet were not totally comparable, the relative amino acid composition was virtually the same since most of the protein was derived from the chow itself It is well known that changes in the ratio of plasma tyrosine to other large neutral amino acids can alter the amount of brain tyrosine although this effect is not clearly related to the amount of tyrosine in the diet [34,35] Furthermore, while dietary tyrosine can affect catecholamine metabolism in the brain, this influence appears to be primarily upon neurons which are in a heightened state of activation and is most marked in dopaminergic neurons [25,33] Since the major effect of diet in the current studies was predominantly upon NE rather than DA metabolism, it appears unlikely that differences in dietary amino acid composition could easily explain the observed changes

In light of the diet-induced changes in NE metabolism seen here in various brain areas, it is interesting that NE does appear to be involved in the regulation of food intake Its most prominent effect is seen in the PVN where it has been shown to stimulate eating in satiated rats through a predominantly α-adrenergic mechanism [11] On the other hand, DA and E appear to have a predominantly inhibitory effect on feeding when given into the perifornical area at the level of the VMH [13] While the PVN was one of the areas in which NE metabolism was increased in DIO and DR rats, this appears to have been a rather non-specific and diffuse effect since similar changes were seen in several other areas of the hypothalamus and even the frontal cortex Although DIO and DR rats may have had similar caloric intakes to chow-fed controls, it is not known how their meal patterns might have differed as regards size and frequency of individ-

ual meals Therefore, some of the observed dietary effects on brain NE metabolism could conceivably have been due to either chronic alterations in the circadian pattern of feeding or to differences in intake during the 3 hr period during which turnover was measured

Perhaps even more intriguing than the diet-induced changes in NE metabolism was the obesity-associated depression of DA turnover seen in the ME and, to a lesser extent, the DMN It is not immediately apparent how changes in these two hypothalamic areas might be related to each other physiologically since the ME receives the majority of its dopaminergic input from the tubero-infundibular DA neurons while the DMN appears to derive its dopaminergic innervation primarily from the periventricular system of DA neurons [26] These 2 hypothalamic nuclei presumably have quite different controlling mechanisms and their rates of DA turnover were very different in the current studies (see Controls, Table 2) One feature common to both the ME and DMN is the relatively high level of thyrotropin releasing hormone found in each area [3] While we have not measured thyroid function in our DIO and DR rats, Rabolli and Martin [29] reported increased plasma T3 and T4 levels in rats fed high fat diets for 4 weeks There are known interactions between thyrotropin releasing hormone and central DA metabolism [27] but it remains unclear what the exact locus of this interface might be This unresolved issue is obviously important given the prominent role played by thyroid hormones in the control of thermogenesis

The act of feeding itself has been shown to activate certain DA neurons in the brain [32] and it is possible that decreased food intake in the DIO rats might have produced the lower DA turnover seen in their brains as compared to chow-fed controls This is unlikely, however, because DR rats generally eat even less than DIO rats [15] yet they showed no decrease in their brain DA turnover here Interestingly, both male and female genetically obese Zucker rats have decreased levels of DA in the DMN at 7 months of age [17] while no differences in ME DA levels were seen in these animals or in 2 month old male or 4 month old female obese Zucker rats [5,6] Obese Zucker rats also show decreased NE levels in the PVN and VMH depending on their age and sex [5, 6, 17] Finally there are no changes in brain DA levels seen in rats with obesity induced by ventromedial hypothalamic lesions [4] Since actual turnover studies have been carried out in neither of these other animal models of obesity, an effect of obesity on DA metabolism cannot be ruled out at present

The major role of DA in the ME appears to be its tonic inhibition of prolactin release [23] While there have been no studies of prolactin metabolism in chronic DIO, genetically obese Zucker rats have abnormalities in their circadian rhythms of serum prolactin levels [24] and genetically obese (obob) mice also appear to have defective prolactin turnover [10,31] Whether or not these abnormalities in prolactin metabolism are in some way causally related to the development and maintenance of obesity remains to be seen although the finding of decreased ME DA metabolism in rats with chronic DIO suggests that further investigation of this question is warranted Of course the major question remains as to whether any of the differences in brain catecholamine metabolism seen in the DIO rats were primarily the cause or effect of their obesity Nevertheless, the finding of major changes in brain catecholamine turnover in an animal model of obesity in which neither multifactorial genetic defects nor brain lesions are present to confound the picture strongly suggests that these changes might be important in the pathogenesis of obesity

ACKNOWLEDGEMENTS

We thank Garry Mackie and Jocelyn Talingdon for their expert technical assistance and Patty Manion and Cathy Muscara for manuscript preparation This work was funded in part by Grant RO 1-AM-3006602 from the National Institutes of Health and the Research Service of the Veterans Administration

REFERENCES

1 Anderson, G H Control of protein and energy intake Role of plasma amino acids and brain neurotransmitters *Can J Physiol Pharmacol* **57** 1043–1057 1979

2 Brodie, B B , E Costa, A Dlabec, N N Neff and H H Snookler Application of steady-state kinetics to the estimation of synthesis rate and turnover time of tissue catecholamines *J Pharmacol Exp Ther* **154**· 493–498, 1966

3 Brownstein, M J , M Palkovits, J M Saavedra, R M Bassire and R D Utiger Thyrotropin-releasing hormone in specific nuclei of rat brain *Science* **185.** 267–269, 1974

4 Coscina, D V , D D Godse and H C Stancer Neurochemical correlates of hypothalamic obesity in rats *Behav Biol* **16** 365–372, 1976

5 Cruce, J A F , N B Thoa and D M Jacobowitz Catecholamines in the brains of genetically obese rats *Brain Res* **101:** 165–170, 1976

6 Cruce, J A F , N B Thoa and D M Jacobowitz Catecholamines in discrete areas of the hypothalamus of obese and castrated male rats *Pharmacol Biochem Behav* **8.** 287–289, 1978

7 Cunningham, J C , L Eisikowitz, W Zawalich and P Felig Increased efficiency of weight gain and altered cellularity of brown adipose tissue in rats with impaired glucose tolerance during diet-induced over-feeding *Diabetes* **32.** 1023–1027, 1983

8 Faust, I M , P R Johnson, J S Stern and J Hirsch Diet-induced adipocyte number increase in adult rats a new model of obesity *Am J Physiol* **235.** E279–E286, 1978

9 Hawrylewicz, E J , H H Huang, E A Drab and J Q Kissane Effect of dietary protein upon hypothalamic biogenic amines in the developing and mature rat *Nutr Rep Internat* **28:** 89–101, 1983

10 Larson, B A , Y N Sinha and W P Vanderlaan Serum growth hormone and prolactin during and after the development of obese-hyperglycemic syndrome in mice *Endocrinology* **98:** 139–145, 1976

11 Leibowitz, S F Paraventricular nucleus a primary site mediating adrenergic stimulation of feeding and drinking *Pharmacol Biochem Behav* **8:** 163–175, 1978

12 Leibowitz, S F , M Jhanwar-Uniyal and B E Levin Effects of amphetamine on catecholamine levels and turnover in discrete hypothalamic areas *Brain Res* **266:** 348–354, 1983

13 Leibowitz, S F and C Rossakis Mapping study of brain dopamine- and epinephrine-sensitive sites which cause feeding suppression in the rat *Brain Res* **172:** 101–113, 1979

14 Levin, B E Retrograde axonal transport of β-adrenoreceptors in rat brain Effect of reserpine *Brain Res* **300**· 103–112, 1984

15 Levin, B E , M B Finnegan, J T Triscari and A C Sullivan Brown adipose and the metabolic features of chronic diet-induced obesity *Am J Physiol* **248:** R717–R723, 1985

16 Levin, B E and A C Sullivan Catecholamine synthesizing enzymes in various brain regions of the genetically obese Zucker rat *Brain Res* **171**· 560–566, 1979

17 Levin, B E and A C Sullivan Catecholamine levels in discrete brain nuclei of seven month old genetically obese Zucker rats *Pharmacol Biochem Behav* **11:** 77–82, 1979
18 Levin, B E and A S Sullivan Dietary obesity and neonatal sympathectomy II Thermoregulation and brown adipose metabolism *Am J Physiol* **247:** R988–R994, 1984
19 Levin, B E , J Triscari and A C Sullivan Altered sympathetic activity during development of diet-induced obesity in rat *Am J Physiol* **244:** R347–R355, 1983
20 Levin, B E , J Triscari and A C Sullivan Relationship between sympathetic activity and diet-induced obesity in two rat strains *Am J Physiol* **245.** R367–R371, 1983
21 Levin, B E , J Triscari, E Marquet and A C Sullivan Dietary obesity and neonatal sympathectomy I Effects on body composition and brown adipose *Am J Physiol* **247:** R979–R987, 1984
22 Lowry, O N Rosebough, A Farr and R J Randall Protein measurement with the Folin phenol reagent *J Biol Chem* **193:** 265–275, 1951
23 McLeod, R M Regulation of prolactin secretion In *Frontiers in Neuroendocrinology*, vol 4, edited by L Martini and J D Ganong New York Raven Press, 1976, pp 169–194
24 Martin, R J , P J Wangsness and J H Gahagan Diurnal changes in serum metabolites and hormones in lean and obese Zucker rats *Horm Metab Res* **10:** 187–192, 1978
25 Melamed, E , F Hefti and R J Wurtman Tyrosine administration increases striatal dopamine release in rats with partial nigrostriatal lesions *Proc Natl Acad Sci USA* **88:** 4305–4309, 1980
26 Moore, R Y and F E Bloom Central catecholamine neuron systems Anatomy and physiology of the dopamine systems *Annu Rev Neurosci* **1:** 129–169, 1978
27 Nielsen, J A and K E Moore Thyrotropin releasing hormone and its analog MK-771 increase the efflux of dihydroxyphenylacetic acid but not 5-hydroxyindolacetic acid into cerebroventricular perfusates of unanesthetized rats *Soc Neurosci Abstr* **9:** 992, 1983
28 Palkovitz, M Isolated removal of hypothalamic or other brain nuclei of the rat *Brain Res* **59:** 449–450, 1973
29 Rabolli, D and R J Martin Effects of diet composition on serum levels of insulin, thyroxin, triiodothyronine, growth hormone and corticosterone in rats *J Nutr* **107·** 1068–1074, 1977
30 Sclafani, A and D Springer Dietary obesity in adult rats Similarities to hypothalamic and human obesity syndromes *Physiol Behav* **17:** 461–471, 1976
31 Sinha, Y N , C B Salocks and W P Vanderlaan Control of prolactin and growth hormone secretion in chemically induced and genetically obese mice *Endocrinology* **97:** 1386–1393, 1975
32 Sved, A F Precursor control of the function of monoaminergic neurons In *Nutrition and the Brain*, vol 6, edited by R J Wurtman and J J Wurtman New York Raven Press, 1983, pp 223–275
33 Sved, A F and J D Fernstrom Tyrosine availability and dopamine synthesis in the striatum Studies with gamma-butyrolactone *Life Sci* **29:** 743–748, 1981
34 Thurmond, J B , N R Kramarcy, S M Lasley and J W Brown Dietary amino acid precursors Effect on central monamines, aggression and locomotor activity in the mouse *Pharmacol Biochem Behav* **12.** 525–532, 1980
35 Thurmond, J B , S M Lasley, N R Kramarcy and J W Brown Differential tolerance to dietary amino acid-induced changes in aggressive behavior and locomotor activity in mice *Psychopharmacology (Berlin)* **66·** 301–308, 1979
36 Triscari, J , C Nauss-Karol, B E Levin and A C Sullivan Changes in lipid metabolism in diet-induced obesity *Metabolism* **34·** 580–587, 1985
37 Wurtman, R J and J D Fernstrom Control of brain monoamine synthesis by diet and plasma amino acids *Am J Clin Nutr* **28·** 638–647, 1975