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Behavioral and Endocrine Effects of Aminodipropionitrile (ADPN) in Male Mice

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SACKLER, A. M., A. S. WELTMAN AND R. SCHWARTZ. *Behavioral and endocrine effects of aminodipropionitrile (ADPN) in male mice.* PHARMAC. BIOCHEM. BEHAV. 1(2) 191–196, 1973.–Effects of aminodipropionitrile (ADPN) on behavior, locomotor activity, food consumption, body weights and endocrine function were noted in male albino mice (CFW). Six daily, consecutive intraperitoneal injections of the drug caused hyperactivity and behavioral phenomena which resembled the circling and headshaking syndrome observed in mutant waltzing mice. Two dose levels 1.0 and 1.5 g/kg were administered to the test groups. Control animals received normal saline. All mice were sacrificed at the end of one week, 48 hr after the 6th and final injection. At both dose levels, the findings revealed significant decreases in body weights, food consumption, food utilization and fecal excretion with consistently more pronounced effects being caused by the 1.5 g/kg dose. Significant increases were noted in the locomotor activity and circling activity of the test mice. Total WBC counts were significantly decreased and adrenal ascorbic acid content/gland significantly increased in the test groups. These findings together with increases in the relative adrenal weights and decreases in the thymus and spleen weights indicated significantly increased pituitary–adrenocortical activity in the test mice. Significant decreases in the seminal vesicle weights of the test vs. control mice suggested hypogonadism. The various data while in accordance with nonspecific stress, also demonstrated certain similarities and parallelisms to behavioral patterns and endocrine activities in the mutant waltzing (whirler) stock.

Male mice Aminodipropionitrile ADPN Endocrine organs Locomotor activity Circling Food consumption Food utilization Fecal elimination Total white blood cell counts Adrenal ascorbic acid

PREVIOUS studies of behavioral, metabolic and endocrine organ characteristics of the female whirler [23,32] and male whirler mutant mice [33] demonstrated consistently decreased body weights, increased metabolic rates and heightened adrenocortical activity throughout the life span of the homozygous whirler mice with indications of hypogonadism. The whirlers [19] represent one of a group of waltzing mice mutations [16] which display syndromes of a rapid, circling locomotor activity, head-shaking and deafness. The mice are extremely restless, nervous and excitable. Neurological abnormalities and anomalies in the central nervous system have been associated with the waltzing type of disorder [16].

Various investigators have employed a variety of compounds to induce similar behavioral and locomotor syndromes which resemble those of the hereditary waltzing strain. Goldin *et al.* [14] have used nitrogen mustard compounds. Delay *et al.* [11], Thuillier and Burger [30], and Rudberg [22] have used aminodipropionitrile (ADPN) and Cutting *et al.* [10], reductones. Goldin *et al.* [14] reported that lesions induced in the cerebellum, brain stem, medulla and upper cord by certain of the nitrogen mustards could account for the chemically-induced neurological waltzing manifestations. Bachhuber *et al.* [3] similarly demonstrated necrosis of the Purkinje cells in the cerebellum and ganglion cells of the anterior horn of the spinal cord in rats administered ADPN. The present study investigated the effects of aminodipropionitrile (ADPN) on behavior, body growth, locomotor activity, food consumption and endocrine function of male albino mice (CFW). The goal was to determine relationships and parallelisms between the behavioral and endocrine characteristics of the chemically-induced versus hereditary waltzing mice.

MATERIALS AND METHOD

Male albino mice (CFW strain, Carworth Inc., New City, New York) averaging 25 g were matched by body weights and divided in two test groups (A and B) and a control group C. Test groups A and B were injected intraperitoneally on 6 consecutive days with 1.0 g/kg and 1.5 g/kg dose levels of aminodipropionitrile (ADPN) in distilled water. These dose levels had been previously used by Rudberg [22] to produce the waltzing syndrome in mice.

Aminodipropionitrile was prepared by the method of Buc *et al.* [4]. ADPN was synthesized under the supervision of Dr. Alex Gringauz, Dept. of Chemistry, Brooklyn College of Pharmacy, Long Island University, Brooklyn, New York. (B. P. 134°–136°C; Sp. Gr. 1.012; $n^{20}D$ 1.4640; M. W. 123.2). Control animals received normal saline injections. Test and control mice were grouped respectively in units of two in circular wire mesh cages having a diameter of 21.5 cm and a height of 17.8 cm. Fine mesh wire screens were placed beneath the floor of the cages to catch and hold fecal pellets as well as food spillage thereby enabling accurate fecal weight and food consumption measurements. The animals were housed in air-conditioned quarters with room temperature maintained at 23–24C. Analyses of sound tests indicated that noise levels averaged approximately 65 db during daylight hours. The number of male mice per cage was limited to 2 animals to minimize fighting and crowding effects [8]. The sensitivity of animal growth and development to such environmental stimuli as temperature [13], noise [24,25], handling [34] and isolation stress [26,36] have been well recognized.

Body weights, food consumption and fecal elimination values were obtained at the completion of the 1-week experimental period. Twenty-four hours after the 6th and final intraperitoneal injection, locomotor and behavioral observations were obtained by modification of open-field testing procedures [1,17] to determine effects of the ADPN dose levels in causing hyperactivity and the waltzing syndrome. Test or control mice were placed centrally on a black painted square board (40.5x40.5 cm). Equally spaced white lines divided the board into 16 squares. The degree of locomotor activity of the control mice was estimated by recording the number of lines crossed by their hind feet during a 20 min observation period using a Veeder-Root hand tally counter. Locomotor and circling activity of the test mice were scored by tabulating each complete circle with one counter and recording the number of lines crossed by their hind feet during an incomplete circle plus the normal line crossing activity with another counter. To estimate and correct for the number of lines crossed while circling, the frequency of circling was multiplied by an arbitrary factor of two and added to the number of lines crossed. This essentially minimal correction factor was utilized since in a few instances depending on the small size of the circle a mouse could whirl without crossing a line but with larger circles cross 4 lines and with still very large circling activity cross 12 lines. Forty-eight hours after the 6th and final intraperitoneal injection and 24 hr after locomotor activity studies, total leukocyte counts were obtained from an average of two tail-blood samples. A standard diluent was used for the total leukocytes [31]. Decreases in total leukocytes have been used to assay adrenocortical function [15,18]. Immediately after tail-blood specimens were obtained, test and control mice were sacrificed by etherization for endocrine and associated organ weight evaluations. The adrenals, thyroids, pituitary, spleen, thymus, liver, testes and seminal vesicles were dissected free of fat and connective tissues and weighed to the nearest 0.1 mg. Adrenal ascorbic acid values were determined for the right adrenals by the method of Mindlin and Butler [20]. Standard *t*-test procedures were used for statistical analyses of the various data [29].

RESULTS

Behavioral and locomotor activity studies 24 hr after the 6th injection demonstrated that all 26 mice in Group B (1.5 g/kg) showed the typical waltzing syndrome of circling and head-shaking patterns. In Group A, all but one mouse showed circling behavior during the 20 min study period. However, at the end of the week, prior to autopsy, the animal showed marked hyperactivity, circling and head-shaking behavior typical of the waltzing syndrome. Table 1 lists the frequencies of lines crossed and circling of test and control mice during the 20 min observation period. Comparisons of the lines crossed by the test and control groups indicated that the 22.0% increase in line-crossing activity of group A (1.0 g/kg) was not statistically significant but that the 1.5 g/kg dose significantly increased the frequency or number of lines crossed. Analyses of the numbers of circles circumscribed by the two test groups indicated a significant increase in the circling activity caused by the larger dose. Totalling and correcting the number of lines crossed by arbitrarily equating each circle to 2 lines revealed significantly increased locomotor activity in both test groups with the greater dose again producing the most marked and significant increase. Table 2 presents body weight, food consumption, fecal elimination and food utilization data of the test and control groups for the 1 week experimental period. Analyses indicated that both dose levels caused significant decreases in the final body weights as well as body weight gains of the test groups with the greater dose producing the more pronounced effects. Similarly, significant decreases were observed in food consumption, fecal excretion and the food utilization or food efficiency ratios (body weight gains/g food consumed) with the greater dose again producing the more pronounced alterations.

Table 3 presents the final body weights and relative endocrine and associated organ weights of the test and control groups sacrificed 48 hr after the 6th injection respectively. In general, the greater dose produced the more pronounced and significant alterations. The 1.0 g/kg dose (Group A) caused significant decreases in the final body weights, relative thymus, spleen and seminal vesicle weights. Conversely significant increases were observed in the relative adrenal and thyroid weights. No significant alterations were noted in the relative pituitary and liver weights. Paradoxically, although significant decreases were observed in the absolute and relative seminal vesicle weights suggestive of hypogonadal effects, a significant increase was noted in the testes weights of the 1.0 g/kg group. With the higher dose th 3.5% increase was not significant. Comparisons on an absolute weight basis indicated weight decreases in the testes of both test groups but only the larger dose caused a significant decrease. The discrepancy between the absolute and relative testes weight data would suggest that the decreases in the respective weights of the testes were not proportionate to the respective or corresponding decreases in the body weights of the test animals. Additional changes caused by the greater dose were significant increases in the relative pituitary and liver weights.

Table 4 presents respectively total leukocyte counts taken several minutes before sacrifice and the adrenal ascorbic acid levels obtained 48 hr after the final injection and at the end of the week study. Comparison of the two test vs. control white blood cell counts revealed significant decreases in the total leukocyte counts of both test groups. Further analyses of Group A and B data indicated a significant decrease in the leukocyte counts of the mice administered the 1.5 g/kg vs the 1.0 g/kg dose.

Analyses of the adrenal ascorbic acid levels revealed an

TABLE 1

EFFECTS OF AMINODIPROPIONITRILE (ADPN) ON LOCOMOTOR ACTIVITY AND CIRCLING BEHAVIOR* OF MALE MICE

	ADPN dose (g/kg)	n	No. Lines Crossed/20 min	No. Circles /20 min	Corrected No. Lines Crossed /20 min
Group A	1.0	18	440.5	48.3	537.2
± S.E.			±58.0	±22.1	±53.0
Group B	1.5	20	575.0	162.4	899.7
± S.E.			±88.1	±26.5	±122.1
Group C	saline	20	361.0	–	361.0
± S.E.			±29.0	–	±29.0
% Diff. (A vs. C)			+22.0	–	+48.8
p Value			0.22	–	<0.01
% Diff. (B vs. C)			+59.3	–	+149.2
p Value			0.03	–	<0.001
% Diff. (A vs. B)			+30.5	+236.6	+67.5
p Value			0.19	<0.01	<0.01

*Activity tested 24 hr after the 6th daily injection.

TABLE 2

EFFECTS OF AMINODIPROPIONITRILE (ADPN) ON BODY WEIGHTS (B.W.), FOOD CONSUMPTION, FOOD UTILIZATION RATIOS AND FECAL EXCRETION OF MALE MICE

				ONE WEEK				
	ADPN dose (g/kg)	n	Initial B.W. (g)	Final B.W. (g)	n*	Food† Consumed (g)	Food Utilization† (B.W. gain/ food consumed	Fecal wt.† (g)
Group A	1.0	23	25.0	22.2	10	57.3	−0.1086	14.79
± S.E.			±0.3	±0.4		±1.9	±0.0182	±1.06
Group B	1.5	24	24.8	20.9	11	52.4	−0.1591	13.28
± S.E.			±0.3	±0.2		±2.6	±0.0273	±0.88
Group C	saline	27	24.8	25.0	12	76.0	+0.0033	22.08
± S.E.			±0.3	±0.5		±2.4	±0.0085	±0.95
%Diff. (A vs. C)			+0.8	−11.2		24.6		−33.0
p Value			0.62	<0.001		<0.001	<0.001	<0.001
% Diff. (B vs. C)			0.0	−16.4		−31.1		−39.9
p Value			>0.90	<0.001		<0.001	<0.001	<0.001
% Diff. (A vs. B)			−0.8	−5.9		−8.6		−10.2
p Value			0.63	0.01		0.16	0.15	0.29

*n = number of paired mice.
†Food consumed, fecal excretion and food utilization ratio calculated on basis of 2 mice per cage per week.

TABLE 3

EFFECTS OF AMINODIPROPIONITRILE (ADPN) ON FINAL BODY WEIGHTS AND RELATIVE ORGAN WEIGHTS OF MALE MICE*

				(g or mg/100 g body weight)								
	ADPN dose (g/kg)	n	Final B.W. (g)	Liver (g)	Spleen (mg)	Thymus (mg)	Adrenals (mg)	Testes (mg)	Sem. Ves. (mg)	Thyroids (mg)	Pituitary (mg)
Group A ± S.E.	1.0	20	21.8 ±0.5	6.32 ±0.16	364.5 ±13.2	63.9 ±5.1	20.1 ±0.6	600.9 ±14.0	130.9 ±5.3	12.1 ±0.7	6.3 ±0.4
Group B ± S.E.	1.5	20	21.0 ±0.3	7.19 ±0.16	360.2 ±26.5	44.0 ±3.6	21.2 ±0.8	566.4 ±25.8	122.3 ±6.1	11.3 ±0.7	7.2 ±0.4
Group C ± S.E.	saline	20	24.8 ±0.5	6.45 ±0.17	493.8 ±39.8	118.9 ±7.4	16.2 ±0.7	547.1 ±16.2	156.3 ±5.3	9.4 ±0.5	5.9 ±0.4
% Diff. (A vs. C)			12.1	-2.0	26.2	-46.3	+24.1	+8.0	-16.3	+28.7	+6.8
p Value			<0.001	0.59	<0.01	<0.001	<0.001	0.05	<0.01	<0.01	0.46
% Diff. (B vs. C)			15.3	+11.5	-27.1	63.0	+30.9	+3.5	21.8	+20.2	+22.0
p Value			<0.001	<0.01	<0.01	<0.001	<0.001	0.54	<0.001	0.04	0.03
% Diff. (A vs. B)			3.7	+13.8	-1.2	-31.1	+5.5	-5.7	-6.6	6.6	+14.3
p Value			0.19	<0.001	0.89	<0.01	0.30	0.26	0.31	0.45	0.13

*Animals sacrificed at the end of one week, 48 hr after the 6th daily injection.

TABLE 4

EFFECTS OF AMINODIPROPIONITRILE (ADPN) ON TOTAL WHITE BLOOD CELLS* AND ADRENAL ASCORBIC ACID† OF MALE MICE

	ADPN dose (g/kg)	n	Total WBC/mm³	n	Right Adrenal Ascorbic Acid (mg/100 mg tissue)
Group A ± S.E.	1.0	20	15698 ±917	20	0.122 ±0.013
Group B ± S.E.	1.5	20	11303 ±964	20	0.136 ±0.011
Group C ± S.E.	saline	20	18488 +951	19	0.090 ±0.015
% Diff. (A vs. C)			-15.1		+35.6
p Value			0.05		0.11
% Diff. (B vs. C)			38.9		+51.1
p Value			<0.001		0.02
% Diff. (A vs. B)			28.0		+11.5
p Value			<0.01		0.42

*Total WBC samples obtained immediately before autopsy at end of one week.
†Adrenal ascorbic acid obtained after autopsy 48 hr after the 6th daily injection.

increase in Group A vs control levels which was not statistically significant. A significant increase was noted in the adrenal ascorbic acid levels of test mice receiving the 1.5 g/kg dose. Comparison of adrenal ascorbic acid levels in terms of mcg/gland, however, indicated significantly higher adrenal ascorbic acid content in both of the test groups.

DISCUSSION

In evaluating the behavioral, physiological and endocrine effects of ADPN, it is apparent that ADPN at both dose levels effectively induced the waltzing syndrome in the experimental mice as tested on the 7th day. The treated mice were significantly hyperactive, showing signs of head-shaking, and circling activity. It should be noted that Azima and Grad [2] reported that subcutaneous daily injections of larger doses (4 g/kg) in mice produced a state of motor excitation, agitation and persistent running around from the third day on. Rudberg [22] administering usually 4 injections of ADPN in dose levels of 0.75–1.5 g/kg intraperitoneally observed hyperactivity and the waltzing syndrome at about the eighth day.

It is evident from the consistent increases in the relative adrenal and pituitary weights accompanied by simultaneously significant decreases in the thymus and spleen weights that ADPN caused heightened activity in the pituitary-adrenocortical axis. Decreases in the thymus and lymphoid organ weights have been used as a measure of increased corticosteroid output [12,27]. Similarly, the significant decreases noted in the total leukocyte counts [15,18] and respective increases in adrenal ascorbic acid levels [28] constitute a measure of stimulated adrenocortical function. Suggestive evidence of inhibited gonadal function stem from the significant decreases in the seminal vesicle weights of both test groups. Despite the significant increases observed in the relative thyroid weights and increased locomotor activity of the test mice, significant decreases were noted in the food consumed by the treated mice. Various investigators have associated levels of thyroid function with changes in food consumption and locomotor activity [21]. The significant increases in the thyroid weights of the test mice might be attributed to possible goitrogenic effects of ADPN. The decreases in body weights, food consumption, food utilization and fecal excretion of the test mice would seemingly suggest dysfunction of food metabolism and utilization processes. The possible debilitating effects on the mice caused by 6 daily injections of ADPN must be considered. It is of interest that in small populations of mice maintained for an additional 19 days after injection, circling frequencies of the ADPN treated mice were increased compared to the previous circling activity recorded on the 7th day of the study.

In correlating similarities produced by chemical induction with ADPN to behavioral, physiological and endocrine differences in hereditary whirlers vs their behaviorally phenotypically normal counterpart (heterozygous whirlers), the following should be noted: whirler female and male mice displayed significantly lower body weights than heterozygous mice and significantly higher adrenocortical activity as indicated by adrenal and lymphoid organ weight changes [23, 32, 33] and decreased white blood cell counts and increased plasma and adrenal corticosterone [35]. Both sexes revealed evidence of hypogonadism [23,35]. Food consumption studies undertaken with female whirler mice, however, presented contrasting evidence of increased food uptake compared to the present ADPN findings [32].

It is evident that exclusive of the behavioral and locomotor activity changes, many of the physiological and endocrine alterations produced by ADPN could be ascribed to nonspecific stress, i.e., decreased body weights, increased adrenocortical activity, decreased lymphoid organ weight and hypogonadism. The results of ADPN stress could agree with forced exercise investigations of male and female chicks [9] and with Christian's population density studies with male and female mice [5, 6, 7]. To avoid biasing effects due to acute ADPN stress and to better correlate and associate physiological and endocrine alterations with the chemical induction of waltzing behavior a longer interval of rest and recovery should be considered in future studies.

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