PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 161, 534–537 (1979)

A Possible Relationship between Brain *N*-Acetylneuraminic Acid Content and Behavior (40591)

BRIAN L. G. MORGAN AND MYRON WINICK

Institute of Human Nutrition, Columbia University, 701 West 168th Street, New York, New York 10032

Among the structural and biochemical changes induced by early malnutrition in the brain are a reduction in the extent of dendritic arborization (1) and a decreased concentration of ganglioside (2) and glycoprotein NANA (3). Our recent work demonstrates that intraperitoneal injections of NANA result in increased brain ganglioside and glycoprotein NANA and prevention of the expected behavioral abnormalities in malnourished animals. Perhaps of even greater interest is that both of these corrections persist in the adult animals. To our knowledge this is the first demonstration that injection of an agent normally found in the brain can both specifically be incorporated at or near its site of action and prevent behavioral abnormalities which would be expected to occur.

Methods. In the study there were 16 littermate pairs of pregnant rats (Sprague–Dawley) selected on the basis of body weight. On the third day of gestation the littermates were randomly distributed into two different groups and housed individually. The first group was then fed, *ad libitum*, a semisynthetic diet containing 200 g casein/kg of diet. For the second group the casein content of the diet was reduced to 100 g/kg. These respective diets were fed to their designated groups throughout the entire period of pregnancy and lactation.

On the third day postpartum the rats were divided into four groups—two undernourished and two well fed—and their litters were reduced to eight pups. From Days 14 to 21 inclusive the pups in one undernourished group and one well-fed group were injected intraperitoneally with 1 mg of NANA/50 g body wt. The other two groups received 1 mg glucose/50 g body wt. The NANA administered was crystalline synthetic material of 95% purity obtained from the Sigma Chemical Company. On Day 21 of lactation 2 male pups from each litter (64 in total) were observed in an open field for 20 min. The open-field was an 80 by 80-cm square area enclosed within wooden walls 60-cm high. The floor was covered with white contact paper and divided into 8-cm squares by black lines. The apparatus was located in a quiet room that was indirectly lighted by a 60-W incandescent bulb. A central area (32 × 32 cm) was designated by squares in the center of the open field. A ball 3.5 cm in diameter was suspended from above to within 2 cm of a corner square ("novel square").

The following behaviors were recorded by an observer during each open-field test: (i) the number of seconds before a rat moved its paws out of the four squares which define the starting corner; (ii) the number of squares traversed by both front and rear paws; (iii) the number of vertical extensions of the head, body, and forelimbs, either free standing or against the wall, excluding those vertical extensions associated with grooming; (iv) the number of times the forelimbs entered the "central area"; (v) the presence or absence of defecation; (vi) the total time the rat passed with its nose in the square over which the object was suspended.

The tested animals were killed by decapitation and the brains removed and analyzed by an investigator with no prior knowledge of the behavioral data in order to minimize bias in the results. Protein was determined by the method of Lowry *et al.* (4), and DNA was extracted from the tissues by the method of Klemperer (5) and estimated by the diphenylamine reaction described by Burton (6). Gangliosides and glycoproteins were extracted from samples of homogenized brain by the Suzuki (7) modification of the method of Folch-Pi *et al.* (8) as adapted by Roukema and Heijlman (9). Total gangliosides were determined as NANA by a modification (Miettinen and Takk-Luukkainen (10)) of the resorcinol method of Svennerholm (11). Glycoproteins were also determined by measurement of the NANA content of the total

0037-9727/79/090534-04$01.00/0

$CHCl_3$:CH_3OH-insoluble residue.

The remaining members of the litters were weaned onto the 20% casein diet and rehabilitated on that diet until 24 weeks of age, at which time 2 male rats from each of the original litters (64 in all) were tested in a Y maze for learning ability. This was an electrified Y maze with a metal grid floor similar to that described by Flexner *et al.* (12). An opaque barrier divided the stem of the Y into a start box and an alley. In order to avoid shock the rat had to move from the start box, down the alley into the correct arm of the maze within 5 sec. Shock was given if after 5 sec the rat had not entered the correct arm of the maze. For half of the rats the right arm was correct and for the remainder the left arm was correct. Each animal was trained at its own shock threshold level. These thresholds were determined individually for each animal using Sechzer *et al.*'s (13) modification of the methods of Evans (14). Training continued until the rat had performed nine consecutive correct responses.

Results. Since the differences among experimental groups for all the activities measured in the open field were basically the same, only the results of one activity will be presented. The results show that the intraperitoneal administration of NANA was associated with profound changes in behavior in the open field. Figure 1 shows that the mean duration of time spent with a novel object placed in the open field of the well-fed control rats was greater than that for the undernourished controls. The administration of NANA was accompanied by a significant increase in both the well-nourished and undernourished rats. Moreover, neurochemical analyses of the brains showed that these changes were accompanied by significant increases in total brain ganglioside and glycoprotein NANA content.

Neurochemical analysis of the pups after open-field testing shown in Table I showed that undernutrition reduces brain cell number and size in both the cerebrum and cerebellum. However, the administration of NANA and its accompanying behavioral changes were not associated with any changes in cell number or size in either undernourished or well-fed rats.

As shown in Fig. 2, the beneficial effects of

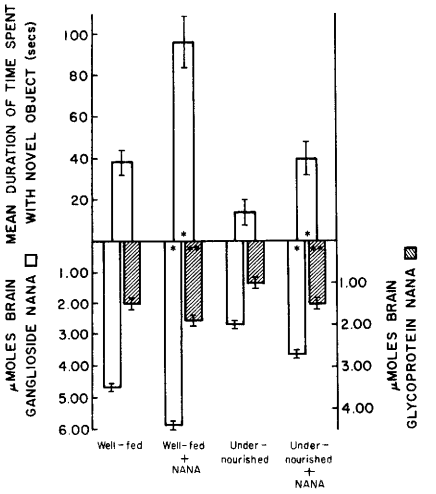

FIG. 1. Open-field behavior in experimental animals of 21 days of age and the corresponding brain content of ganglioside and glycoprotein NANA. Bars represent ($\bar{x}$ ± SE) of values obtained from analysis of 16 separate brains. Where indicated the differences between control and experimental groups were statistically significant ($^*P < 0.001$; $^{**}P < 0.01$). (Differences between control groups and their corresponding undernourished groups were all statistically significant, $P < 0.001$).

NANA administration remained through adulthood. At 6 months of age, treated animals learned the Y maze significantly quicker than their controls. Once again the results of the biochemical analyses revealed that they also had higher total brain ganglioside and glycoprotein NANA contents.

Once again the results of the biochemical analysis, Table II, revealed that the underfed rats rehabilitated after the brain growth spurt did not achieve the same brain size as the well-fed rats. At 24 weeks of age, they still have a smaller cerebral and cerebellar cell number and size. The early administration of NANA was neither associated with an increased cell number or size.

Discussion. A number of workers have proposed a role for sialocompounds in the facilitation and functional establishment of synaptic pathways (15, 16). Schengrund and her co-workers have postulated a role for NANA in effecting the movement of neurotransmitters (17). Bretscher (18) and others (19) have implicated the negative charge associated with NANA in the process of binding neurotransmitters to membranes.

There is a considerable amount of recent

TABLE I. BRAIN COMPOSITION AT 21 DAYS OF AGE ($\bar{x} \pm$ SE)

Group (n = 16)	DNA (mg)	Protein (mg)	Protein/DNA ratio	
Well fed	1.14 ± 0.08	51.65 ± 2.34	45.31 ± 3.14	Cerebrum
Well fed + NANA	1.10 ± 0.07	52.02 ± 4.91	47.29 ± 4.14	
Undernourished	0.93 ± 0.06	40.22 ± 2.5	43.25 ± 5.31	
Undernourished + NANA	0.95 ± 0.07	42.51 ± 2.5	44.75 ± 4.76	
Well fed	0.43 ± 0.06	9.59 ± 0.33	22.31 ± 2.01	Cerebellum
Well fed + NANA	0.48 ± 0.05	10.41 ± 1.12	21.69 ± 1.35	
Undernourished	0.31 ± 0.07	7.86 ± 0.54	25.34 ± 1.71	
Undernourished + NANA	0.34 ± 0.08	8.47 ± 0.62	24.91 ± 2.14	

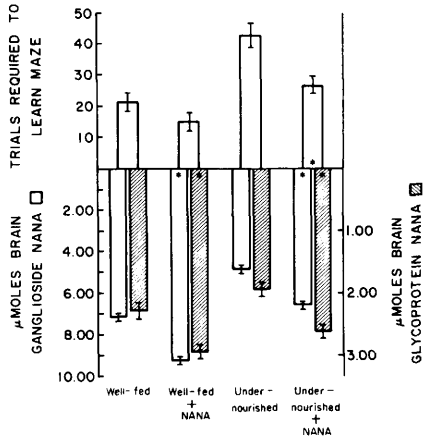

FIG. 2. Y-maze performance in adult animals and the corresponding brain content of ganglioside and glycoprotein NANA. Bars represent ($\bar{x} \pm$ SE) of values obtained from analysis of 16 separate brains. Where indicated the differences between control and experimental groups were statistically significant (*$P < 0.001$). (Differences between control groups and their corresponding under-fed groups were all statistically significant, $P < 0.001$).

evidence to link gangliosides to behavior. Small changes in ganglioside metabolism in the whole brain of rats have been found after short-term behavioral stimulation (20, 21). Passive avoidance learning has been shown to be inhibited by antiserum to brain gangliosides (22). It has been shown that the increase of the content of certain ganglioside fractions in particular areas of the rat brain after long-term active avoidance conditioning, possibly result in permanent functional connections of the neurons involved (23).

Our results clearly show that undernutrition early in life will reduce brain NANA concentrations and that this change will persist. Undernutrition early in life will also produce certain behavioral abnormalities which persist. From earlier studies we know that i.p.-injected NANA is preferentially taken up by the brain during its growth spurt (unpublished results). These studies using ^{14}C-labeled NANA showed that during the time period of 14–21 days postpartum the brain took up and incorporated 10 times as much NANA as the liver, kidney, or spleen. In the same study it was found that ^{14}C-labeled NANA was more readily incorporated into the brains of well-fed control pups than into the brains of undernourished pups.

Here i.p. injection of NANA at this time increased brain ganglioside and glycoprotein NANA concentrations in both the well-fed and undernourished animals. It also was associated with an improvement in the behavioral performance shown by both groups of animals. Furthermore, this improvement was permanent and persisted into adulthood.

This change in behavioral characteristics observed in the experimental groups may not be indicative of a true improvement of brain

TABLE II. BRAIN COMPOSITION AT 24 WEEKS OF AGE ($\bar{x} \pm$ SE)

Group (n = 16)	DNA (mg)	Protein (mg)	Protein/DNA ratio	
Well fed	1.53 ± 0.12	79.81 ± 3.57	52.16 ± 3.16	Cerebrum
Well fed + NANA	1.54 ± 0.14	82.51 ± 4.16	53.57 ± 4.79	
Undernourished	0.96 ± 0.11	49.03 ± 2.51	51.07 ± 2.92	
Undernourished + NANA	0.98 ± 0.15	47.51 ± 3.65	48.48 ± 3.91	
Well fed	0.48 ± 0.07	13.99 ± 0.56	29.15 ± 1.58	Cerebellum
Well fed + NANA	0.47 ± 0.09	13.48 ± 0.43	28.68 ± 2.51	
Undernourished	0.33 ± 0.05	10.65 ± 0.74	32.27 ± 2.72	
Undernourished + NANA	0.34 ± 0.04	10.83 ± 0.89	31.85 ± 2.94	

function. For instance the administration of NANA may principally enhance activity levels which could account for both the open-field and Y-maze results. Nevertheless, whether the NANA causes the changes in behavior directly or via a change in activity does not detract from the interesting association between NANA and behavior. Thus in conclusion we feel that these findings indicate that the impaired metabolism of NANA may play a crucial role in the behavioral abnormalities induced by early malnutrition.

Summary. Repeated intraperitoneal injections of *N*-acetylneuraminic acid (NANA) into malnourished and well-fed rats during the brain growth spurt were associated with a permanent increase in NANA concentrations in brain gangliosides and glycoproteins. Further, there was an alleviation of some expected behavioral abnormalities in the malnourished group and an above normal behavioral performance shown by the well-fed pups. The results suggest the existence of a relationship between NANA and behavior.

1. Salas, M., Diaz, S., and Nieto, A., Brain Res., **73,** 139 (1974).
2. Merat, A., and Dickerson, J. W. T., Biol. Neonate **25,** 158 (1974).
3. Morgan, B. L. G., and Naismith, D. J., Brit. J. Nutr., in press.
4. Lowry, D. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).
5. Klemperer, G., *in* "Methods of Biochemical Analysis" (D. Glick, ed.), p. 287. Interscience, New York (1963).
6. Burton, K. J., Biochemistry **62,** 315 (1956).
7. Suzuki, K., Life Sci. **3,** 1227–1233 (1964).
8. Folch-P. J., Lees, M., and Sloane-Stanley, G. H. S., J. Biol. Chem. **226,** 497 (1957).
9. Roukema, P. A., and Heijlman, J., J. Neurochem. **17,** 773–780 (1970).
10. Miettinen, T., and Takk-Luukkainen, I. T., Acta Chem. Scand **13,** 856 (1959).
11. Svennerholm, L., Biochem. Biophys. Acta **24,** 604 (1957).
12. Flexner, J. B., Flexner, L. B., and Stellar, E., Science **141,** 57 (1963).
13. Sechzer, J. A., Turner, S. G., and Liebelt, R. A., Psychon. Sci. **4,** 259 (1966).
14. Evans, W. O., Psychol. Pharmacol. **2,** 318 (1961).
15. Barondes, H. S., *in* "The Neurosciences" (G. C. Quarton, I. Melnechuck, and F. O. Schmitt, eds.), p. 47. The Rockefeller University Press, New York (1970).
16. Bogoch, S., Adv. Exp. Med. Biol. **71,** 233 (1976).
17. Schengrund, C. L., and Nelson, J. T., Biochem. Biophys. Res. Commun. **62,** 217 (1975).
18. Bretscher, M. S., Science **181,** 622 (1973).
19. Weseman, W., FEBS Lett. **3,** 80 (1969).
20. Dunn, A. J., and Hogan, E. L., Pharmacol. Biochem. Behav. **3,** 605 (1975).
21. Irwin, L. N., and Simonson, F. E., J. Neurochem. **18,** 203 (1971).
22. Karpiak, S. E., Graf, L., and Rapport, M. M., *in* "Neuroscience Abstracts 443." Sixth Annual Meeting, Society for Neurosciences, Bethesda, Md (1976).
23. Savaki, H. E., and Levis, G. M., Pharmacol. Biochem. Behav. **7,** 7 (1977).

Received February 28, 1979. P.S.E.B.M. 1979, Vol. 161.