Effects of a Commercial Soy Lecithin Preparation on Development of Sensorimotor Behavior and Brain Biochemistry in the Rat

JOANNE M. BELL
Department of Psychology
Washington University
St. Louis, Missouri

PAULA K. LUNDBERG
Department of Psychology
University of Texas at Tyler
Tyler, Texas

Pregnant rat dams and offspring were exposed to a 5 or 2% soy lecithin preparation or a control diet. Enrichment was either lifelong beginning at gestation, limited to the time preceding, or the time following weaning, or absent (constituting a "pure" control group). The most marked early sensorimotor deficits (reflex righting and swimming development) were seen in the 5% soy lecithin preparation group, although all soy lecithin preparation-exposed offspring had elevated brain/body weight ratios and choline acetyltransferase levels. Later, animals exposed to lifelong 5 or 2% soy lecithin preparations were hypoactive, had poor postural reflexes, and showed attenuated morphine analgesia. The results indicate that dietary soy lecithin preparation enrichment during development leads to behavioral and neurochemical abnormalities in the exposed offspring.

In the past few years, evidence has accumulated suggesting that levels of acetylcholine (ACh) might be influenced by precursor availability: The brain lacks the ability to synthesize choline *de novo* (Cohen & Wurtman, 1976), and both uptake of choline and the synthesis of ACh require high concentrations of their substrates to reach full saturation (Haubrich, Wang, Chippendale, & Proctor, 1976). Consequently, dietary administration of the ACh precursor, choline, produces elevation in ACh (Haubrich, Gerber, & Pflueger, 1979). These observations have led to the experimental treatment of a number of psychiatric disturbances characterized by deficient cholinergic tone with choline chloride or purified lecithin (Bartus, Dean, Beer, & Lippa, 1982). The use of lecithin has also been suggested for the treatment of hypercholesterolemia and the hyperlipidemias (Assman & Brewer, 1974). Nontherapeutic functions ascribed to commercially available

Reprint requests should be sent to Joanne M. Bell, Department of Pharmacology, 436 Nanaline H. Duke Building, Duke University Medical Center, Durham, North Carolina 27710, U.S.A.

Received for publication 7 March 1984
Revised for publication 23 July 1984
Developmental Psychobiology, 18(1):59-66 (1985)

CCC 0012-1630/85/010059-08$04.00

soy lecithin preparations (SLP) by food faddists include its "fat mobilizing" properties for weight loss and cholesterol reduction and represent a potential for uncontrolled intake of these substances. Neurotransmitters such as ACh have also been implicated as trophic factors in development (Prives & Quastel, 1969), and lecithin given to prenatal or neonatal animals is rapidly incorporated into brain tissues either as membrane constituents (Davidson & Dobbing, 1969), ACh (Atterwill & Prince, 1978), or both. The prescribed and nonprescribed uses of commercially available SLP may thus become critical during pregnancy. The present investigation was designed to assess the possibility that the presence of SLP during brain development could alter basic sensorimotor and neurological maturation in the perinatal rat.

Method

Two weeks before breeding, 40 primiparous Sprague-Dawley female rats were placed ad libitum on a control or experimental 2 or 5% SLP diet until weaning of their litters (control, 2% PRE, 5% PRE diets). The control diet was an AIN 76 standard pelleted lab chow, containing .09% choline (ICN Nutritional Biochemicals). The experimental diets (oils removed) were composed by modifying the control diet as follows: addition of SLP (2 or 5% by weight) with the deletion of equal amounts of sucrose by weight; total choline content was .14% for the 2% SLP diet, and .22% for the 5% SLP diet. The SLP was composed of 79% acetone insolubles (or phosphatides), of which 25% was phosphatidylcholine, 22% phosphatidylethanolamine, 16% phosphoinositol, 5% phosphatidic acids, and 9% polar lipids, ash, sugars, sterols, moisture, etc. Based on a food consumption of 10 g/day, animals were receiving either 8.9 (control), 14.0, or 22.0 mg of choline per day. After weaning, offspring with either maintained on their respective diets, constituting the 2% PRE/POST and 5% PRE/POST SLP diets; or half of the animals that previously received the control diet, were then placed on an enriched diet (2% POST and 5% POST SLP diets), while the other half remained on the same control diet ("pure" control diet). Animals were housed individually during breeding and with litters during preweaning, four same sex-same treatment groups per cage following weaning in a light and temperature controlled animal housing chamber. Only litters with at least eight pups and with nearly equal sex distributions were used. Equal number of both sexes were randomly selected from each litter for sensorimotor, behavioral or biochemical testing.

Behavioral Data

A maximum of two male and two female animals from each litter contributed to each behavioral measure. Maternal behavior was observed on postnatal (PN) days 3, 7, 15, and 19 for 1 hr, according to the procedure described by Crnic (1976). A developmental test battery designed to evaluate neurobehavioral toxicity in rats (Vorhees, Butcher, Brunner, & Sobotka, 1979) was administered PN days 3–20, and included the righting (PN days 3–7), negative geotaxis (PN days 6–12), pivoting (PN days 7, 9, 11), and startle responses (PN days 10–14), as well as development of swimming behavior (PN days 6–20). On the preweaning tests, all animals received the entire battery; the maximum number of subjects per dietary condition was 48 animals. Postweaning measures examined included indices of motor activity (PN day 43), postural reactions (PN days 44–46), pain sensitivity to electrical foot shock (PN day 47), active (PN days 48–52) or passive avoidance learning (PN days 51–52), and morphine-induced analgesia (PN days 47–53). For the postweaning measures, all animals were assessed for motor activity, postural reactions, and

pain sensitivity. Animals were then randomly divided into those that received active avoidance or passive avoidance testing. Twenty-four hr after reaching a criterion of performance on either avoidance test, morphine analgesia was assessed. The maximum number of subjects per dietary condition for the postweaning measures was 16 animals. Brain and body weight ratios were taken on PN days 1, 21, 42, and 67.

Biochemical Data

A maximum number of two male and two female animals from each litter were used in biochemical testing. All biochemical analyses were conducted on behaviorally naive animals. The enzyme choline acetyltransferase (CAT) was assayed in whole brains of PN day-1 animals, and in forebrain (anterior to a cut made at the mesencephalic-diencephalic junction) sections for all other days. CAT analysis was performed using the Dowex assay method as described by Schrier and Schuster (1967) and modified as follows: Tissue was homogenized in isotonic KCl and the incubation mixture included 60 ul of $NaPO_4$ buffer, which contained 10 ul of 2.0 n*M* ^{14}C-acetyl CoA (New England Nuclear). Enzyme blanks were obtained either by adding (1,2 napthylvinylpyridine hydrochloride, Betz Chemicals) or by deleting choline from the duplicate samples; activity was always at least 5 times blank values.

Prior to statistical comparison, all dependent variables were examined for skewing, litter concordance, and sex interactions. An overall omnibus *F* or multivariate ANOVA was first computed on all behavioral or biochemical variables to avoid Type II error, which would occur with multiple comparisons. If significant differences were found, data were repeated, measures were then subject to a Treatment x Age ANOVA, or a simple one-way ANOVA. Post hoc tests were conducted using Scheffé's technique (Winer, 1971).

Results

Dietary enrichment with SLP had a profound effect on early sensorimotor development, demonstrated most distinctly by an increase in the latency to right. As seen in Figure 1, this effect was most marked in the 5% SLP enriched groups. These results thus indicate a disruption of early motor coordination with high levels of SLP enrichment.

To examine whether perturbation of motor control would persist past the early righting stage, the ontogeny of swimming development was examined. Analysis of variance was computed on four blocks of swimming days: PN 6-8, when the pup floats or swims in circles with the nose at or below water level; PN 9-12, when body angle changes so that the head is maintained above water level; PN 13-16, when forward locomotion develops and permits swimming in a straight line; and PN 17-20, when an adult pattern (forelimb inhibition) emerges. Significant effects on swimming development were found for PN days 9-12 and 13-16, as shown in Figure 2. The swimming deficit of the 5% SLP group was primarily a function of the pups keeping their noses partially submerged for a longer period of time. Less critical was the delayed development of both forward movement and extent of paddling. This latter aspect may have been a carryover from the delayed development of nose position. Thus, it was apparent that high levels of SLP enrichment caused alterations in multiple forms of motor behavior, and persisted into the late preweaning period.

But, are these lecithin-induced changes found only in relation to motor coordination? Results of the swimming task indicate that more integrated adult forms of behavior may also be affected. Postweaning behavioral measures indicated that animals on lecithin SLP

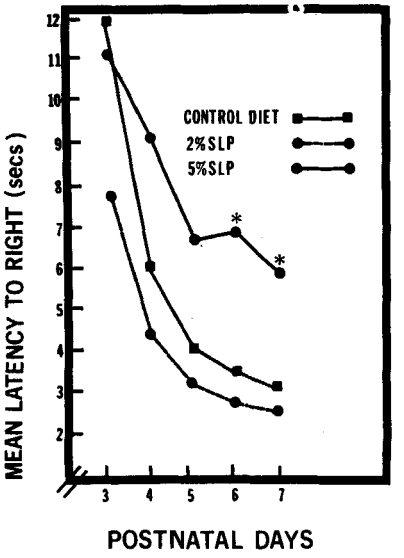

Fig. 1. Effects of SLP on surface righting response as a function of age in days. Data points are averaged over 2 trials/day (30-sec criteria). (Treatment × age, $p < .01$; Scheffé post-hoc* $p < .01$.)

the longest (PRE/POST diets) were significantly less active than other dietary groups, as measured by open-field tests (mean number of rearing responses in a 3-min period). While high levels of dietary SLP enrichment resulted in hypoactivity in an open-field situation, these effects were not generalized to all activity or avoidance measures. Although there were no differences in either active or passive avoidance tasks, performance on the neurological battery, an indication of the functional state of motor control by the cerebral-cerebellar axis, was disrupted in animals receiving high levels of SLP. The results of this and the open-field tests are illustrated in Table 1.

The actions of cholinergic agonists on nociception are of potential significance in the development of tolerance and addiction to opiates, and parenteral and intravenous administration of cholinomimetics have been shown to potentiate morphine-induced analgesia (Metys, Wagner, Metysova, & Herz, 1969). In the current study, both drug-free and drug-

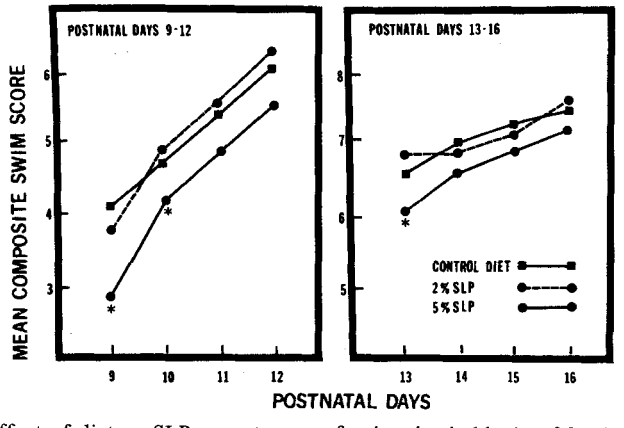

Fig. 2. Effect of dietary SLP on ontogeny of swimming in blocks of 9–12 (diet, $p < .0001$; diet × age, $p < .0001$) and 13–16 days of age (diet, $p < .0001$; diet × age, $p < .0001$). Scheffé post-hoc* $p < .05$). Data points are composite scores of head angle, direction, and limb use.

TABLE 1. Means (+/-SE) of Postweaning Behavioral Tests.

Group	Open-field Scores[a]	Neurological Tests	
		Hindlimb Placing[b]	Forelimb Hopping[c]
Control	30 ± 4	2.3 ± .2	3.5 ± .2
2% SLP (PRE)	27 ± 3	3.0 ± .2	3.6 ± .2
2% SLP (POST)	31 ± 3	3.1 ± .2*	3.8 ± .1
2% SLP (PRE/POST)	23 ± 7*	2.9 ± .2	3.8 ± .1
5% SLP (PRE)	35 ± 2	3.1 ± .2*	3.6 ± .1
5% SLP (POST)	28 ± 4	1.8 ± .2	3.1 ± .2*
5% SLP (PRE/POST)	29 ± 4	2.9 ± .2	3.8 ± .1

[a]Diet, $p < .001$.
[b]Diet, $p < .0001$.
[c]Diet, $p < .0011$.
*Scheffé post-hoc $p < .05$.

induced nociceptive responses were examined. Univariate analysis conducted on foot-shock current (mA) required for elicitation of flinch and jump responses revealed no significant differences among dietary conditions. However, analysis of tail-flick latency following morphine administration, as shown in Figure 3, indicated an antagonistic effect of SLP on morphine analgesia. To corroborate that this effect was due to an opiate mechanism, the ability of naloxone to antagonize the morphine analgesia was examined, and found to affect all groups similarly. The result of both the flinch-jump tests and the pre-morphine tail-flick data also showed that dietary SLP had no analgesic effect on its own.

To examine whether these behavioral changes were due either to direct or indirect mechanisms, several morphological and neurochemical measurements were undertaken. Specific change within the cholinergic system was measured by analysis of choline acetyltransferase levels at various ages (Table 2). Although CAT is not the rate-limiting step in

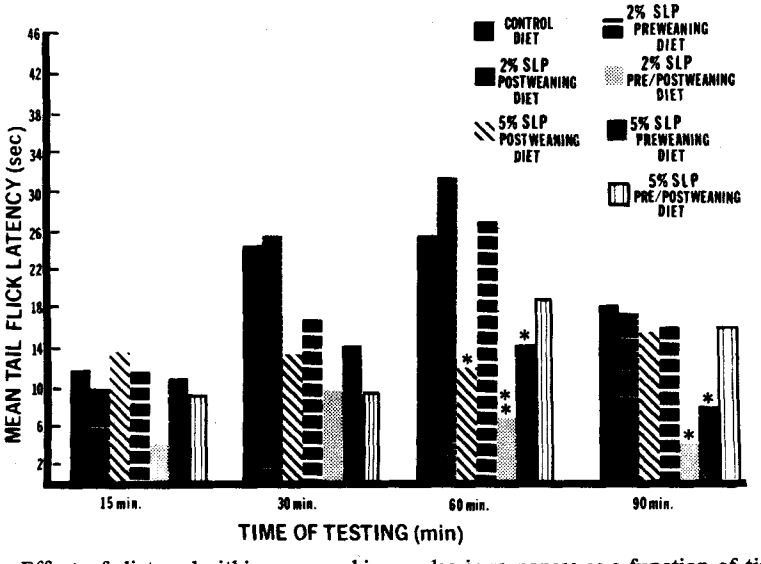

Fig. 3. Effect of dietary lecithin on morphine analgesic responses as a function of time. Data points are latency to tail-flick following 3 mg/kg morphine sulfate administration (s.c.). (Diet, $p < .005$; diet X time, $p < .01$; Scheffé post-hoc** $p < .01$, *$p < .05$.)

TABLE 2. Choline Acetyltransferase Levels (Mean +/- SE) for all Dietary Groups.[a]

Group	Postnatal Age (days)			
	1	21	42	67
Control	12 ± 2	12 ± 2	34 ± .3	52 ± 4
2% SLP (PRE)	18 ± 2*	15 ± 2	45 ± 1.5	52 ± 3.5
2% SLP (POST)	–	–	40 ± 6	49 ± 4
2% SLP (PRE/POST)	–	–	35 ± .6	46 ± 3
5% SLP (PRE)	23 ± 2*	22 ± 2*	48 ± 7*	49 ± 3
5% SLP (POST)	–	–	43 ± 10	53 ± 5
5% SLP (PRE/POST)	–	–	45 ± 9*	54 ± 4

[a]CAT activity is expressed as CPM/n*M*/mg/hr. Preweaning analysis: diet, $p < .01$; Scheffé* $p < .05$. Postweaning analysis: diet, $p < .05$, Scheffé* $p < .05$.

acetylcholine synthesis, it is a widely used indicator of the changes occurring in the cholinergic system during ontogenetic development. It appeared that dietary SLP given during perinatal life resulted only in an early elevation in CAT activity, above and beyond the expected maturation of the enzyme. These results suggest that dietary SLP modifies cholinergic function in some manner. Be that as it may, this alteration may not be relevant to cholinergic function. Indeed, the effect on CAT may reflect more generalized alterations in the SLP exposed offspring. Further, examination of brain/body weight ratios indicates that such a nonspecific mechanism may be operating to produce behavioral alterations. Analysis of preweaning brain/body weight ratios indicated an effect of the SLP (diet, $p < .003$; diet x age, $p < .02$), and that the 2% SLP group on PN day 1 had a larger ratio than did the control group (Scheffé, $p < .05$). Postweaning analysis again indicated that the SLP had an effect on brain/body weights (diet, $p < .001$), with animals exposed to either high (5% SLP) or chronic (PRE/POST) levels of SLP to have higher ratios than control animals in this index (Scheffé, $p < .05$) on PN day 42. It is unlikely that these morphological changes would be related to subtle modifications in cholinergic function.

Finally, to assess whether these behavioral, morphological, and biochemical alterations were due indirectly to changes in maternal caretaking as a function of the diet, time-sampling of nursing behavior was conducted. There were no differences in maternal care among the groups, nor was there any dietary rejection or anorexia exhibited by the dams fed the SLP diet; indeed, SLP-enriched animals had higher body weights (diet x day, $p < .0001$), on PN days 14 and 21 (Scheffé, $p < .05$) than control animals.

Discussion

This study demonstrates that a SLP-supplemented diet fed to rat dams and their offspring results in alterations of sensorimotor development and of transmitter neurochemistry. Administration of SLP during the perinatal period produced delays in the development of both reflex righting and swimming behavior. During the early juvenile period, rats exposed to either chronic or high levels (or both) of SLP during development were hypoactive, showed impaired hindlimb neurological responses and attenuated morphine analgesia. Since sensorimotor as opposed to avoidance (e.g., "cognitive") tasks were disrupted, SLP may be exerting its major effect on cerebellar rather than cortical growth,

perhaps by virtue of their regional developmental patterns in conjunction with timed exposure. Evidence (in preparation) supports the hypothesis of regional specificity in terms of SLP effects on cellular maturation.

But, do these data imply that the behavioral abnormalities involve a specific cholinergic mechanism, or instead, are simply an expression of generalized perturbation of cellular processes? In argument of the first alternative, SLP enrichment resulted in an increase in levels of CAT activity during early life. Although such increases are not sustained beyond sexual maturity, it suggested that precursor loading could accelerate normal maturation of this enzyme system (Tucek, 1978). However, the most relevant measure of change in ACh levels would be best measured by amount of ACh turnover; therefore, increases in CAT levels may not have directly paralleled ACh levels in brain.

Additionally, the present results are similar to those found in animals exposed to 3% phenylalanine enrichment in the maternal diet, viz., hypoactivity and delays in the appearance of the surface righting reflex and in the onset of an adult pattern of swimming (Brunner, Vorhees, Kenny, & Butcher, 1979). The observation that the modulation of precursor availability with two different neurotransmitter systems results in similar behavioral abnormalities suggests that a more generalized change in the brain biochemistry of the SLP-exposed animals was taking place.

It is highly likely that the observed behavioral alterations may be secondary to a generalized change in cellular maturation occurring in the developing animal. The present results do not lend support to predictions based on SLP effects solely on the cholinergic system: Although other cholinergic agents have been previously reported to depress ongoing motor activity, thereby facilitating the acquisition of a passive avoidance task (Meyers, 1965), the hypoactivity following the SLP did not have such an effect on this task. Similarly, SLP effects on morphine analgesia diverged from what would have been predicted based on previous findings of facilitatory effects of cholinergic agonists (Van Eick & Bock, 1971). In this respect, the present results are similar to only one recent study whose authors suggest that morphine's acute analgetic actions involve the suppression of ACh release, and agents that increase central cholinergic activity should attenuate morphine-induced analgesia (Botticelli, Lytle, & Wurtman, 1977). Finally, SLP enrichment resulted in changes of the brain/body weight ratio in exposed offspring. These results may be related to the finding that a large proportion of the phospholipids taken across the immature blood-brain barrier may be rapidly incorporated into cell constituents, thereby modifying general development (Cornford, Braun, & Oldendorf, 1978).

In conclusion, these data indicate that precursor loading may be seriously detrimental to the functional development of the exposed offspring. Nontherapeutic use of SLP in the lay population may be predisposing unborn progeny to subtle neurological and biochemical impairments by alteration of a generalized cellular maturation.

Notes

This research was supported by NINCDS Grant NS-11002 to Dr. Stanley Finger. This article was presented in part at a meeting of the Society for Neurosciences, November, 1983 [*Neurosci. Abstr.*, *9*: 151.1 (1983)]. It is part of the Ph.D. dissertation of Joanne M. Bell. The help of Drs. T. A. Slotkin, S. Finger, and E. Johnson in the preparation of this manuscript is gratefully acknowledged.

References

Assman, G. and Brewer, H. (1974). A molecular model of high density lipoproteins. *Proc. Natl. Acad. Sci. USA*, *71*:1534–1538.

Atterwill, C., and Prince, A. (1978). Multiple forms of choline acetyltransferase and acetylation of choline in the rat brain. *Br. J. Pharmacol., 62*:398.

Bartus, R., Dean, R., Beer, and Lippa, A. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science, 217*:408–417.

Botticelli, L., Lytle, L., and Wurtman, R. (1977). Choline induced attenuation of morphine analgesia in the rat. *Comm. Pharm., 1*:519–523.

Brunner, B., Vorhees, C., Kenney, L., and Butcher, R. (1979). Aspartame: Assessment of developmental psychotoxicity of new artificial sweetener. *Neurobeh. Tox., 1*:79–86.

Cohen, E., and Wurtman, R. (1976). Brain acetylcholine: Control by dietary intake. *Science, 191*:561–562.

Cornford, E., Braun, J., and Oldendorf, S. (1978). Carrier-mediated blood brain barrier transport of choline and certain choline analogs. *J. Neurochem., 30*:299–308.

Crnic, L. (1976). Models of infantile malnutrition in rats: Effects on maternal behavior. *Dev. Psychobiol., 13*:615–627.

Davidson, A. and Dobbing, J. (1969). Phospholipid metabolism in nervous tissue: Metabolic stability. *Biochem. J., 75*:565–570.

Haubrich, D., Wang, P., Chippendale, T., and Proctor, E. (1976). Choline and acetylcholine in rats: Effects of dietary choline. *J. Neurochem., 27*:1305–1313.

Haubrich, D., Gerber, N., and Pflueger, A. (1979). Choline availability and the synthesis of acetylcholine. In A. Barbeau, J. Growdon, and R. Wurtman (eds.), *Nutrition and the Brain. Volume 5.* New York: Raven Press. Pp. 57–72.

Metys, P., Wagner, S., Metysova, M., and Herz, S. (1969). Studies on the central antinociceptive action of cholinomimetic agents. *Intern. J. Pharmacol., 8*:413–425.

Meyers, G. (1965). Some effects of scopolamine on passive avoidance response in rats. *Psychopharmacologia, 5*:289–291.

Prives, C., and Quastel, J. (1969). Effects of cerebral stimulation on the biosynthesis *in vitro* of nucleotides and RNA in the brain. *Nature, 221*:1053.

Shrier, B., and Schuster, L. (1967). A simplified radiochemical assay for choline acetyltransferase. *J. Neurochem., 14*:977–985.

Tucek, S. (1978). *Acetylcholine Synthesis in Neurons.* New York: Wiley.

Van Eick, A., and Bock, J. (1971). Comparison of analgesic, cholinomimetic, anticholinergic and sympathomimetic drugs by means of the hot plate. *Arch. Int. Pharmacodyn. Ther., 189*:384–387.

Vorhees, C., Butcher, R., Brunner, R., and Sobotka, T. (1979). A developmental test battery for neurobehavioral toxicity in rats: A preliminary analysis using monosodium glutamate, calcium carrageenan and hydroxyuria. *Toxicol. Appl. Pharmacol., 50*:267–282.

Winer, B. (1971). *Statistical Principles in Experimental Design.* St. Louis: McGraw-Hill.