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Chronic Choline Supplementation Attenuates the Behavioral Effects of Pentobarbital

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WECKER, L., S. ROTHERMEL AND G. CAWLEY. *Chronic choline supplementation attenuates the behavioral effects of pentobarbital.* PHARMACOL BIOCHEM BEHAV **28**(4) 469–475, 1987.—The behavioral and neurochemical effects of pentobarbital were investigated in rats maintained for 28–35 days on a standard choline-containing diet or on a diet containing 10 times the concentration of choline present in standard rodent chow. The supplemented dietary regimen increased the concentration of free choline in serum by 52%, but did not alter the steady-state concentrations of either choline or acetylcholine in brain. Choline supplementation attenuated both the sedative/hypnotic and hypothermic effects of pentobarbital through an action that could not be attributed to either an enhanced peripheral metabolism of pentobarbital or to an attenuation of the cholinergic effects of pentobarbital. Rather, results indicate that chronic supplementation with choline increases cerebral glucose metabolism and causes a behavioral hyperactivity, effects that may mediate the attenuation of the behavioral response of pentobarbital.

Choline supplementation Glucose metabolism Behavioral hyperactivity

CLINICAL interest in the use of choline (or lecithin) for the treatment of neuropsychiatric disorders postulated to involve hypocholinergic activity began shortly after publication of initial studies indicating that acute choline administration increases the levels of acetylcholine (ACh) in brain [3,9]. Although there is an abundance of data, albeit controversial, on the effects of acute choline supplementation, clinical interest involves administration on a chronic basis, the consequences of which are incompletely characterized.

We have previously reported that although dietary supplementation with choline increases circulating levels of choline, it does not alter the steady-state levels of either choline or ACh in brain [26,27]. However, choline supplementation does prevent the depletion of ACh induced by compounds that increase cholinergic neuronal firing, an effect that has been attributed to the enhancement of ACh synthesis during drug-induced increases in neuronal demand [26,27]. Recently, during the course of a series of experiments on choline supplemented rats that required sedation, we observed that our standard anesthetic dose of pentobarbital was not always sufficient to induce surgical anesthesia in these animals. Since the barbiturates decrease the synthesis and release of ACh (for review see [16]), it was not readily apparent why choline supplemented animals were resistant to the anesthetic effects of pentobarbital. Therefore, the main objectives of these studies were to: (1) quantitate the effects of chronic supplementation with choline on the behavioral actions of pentobarbital; and (2) investigate possible neurochemical mechanisms involved. Results indicate that chronic supplementation with choline in the diet attenuates both the sedative/hypnotic and hypothermic effects of pentobarbital. These actions do not appear to be mediated by a direct alteration of the cholinergic effects of pentobarbital, but rather, may be secondary to a neurochemical and behavioral hyperactivity induced by chronic supplementation with choline.

METHOD

Male Sprague Dawley rats (140–160 g initial weight, Harlan Industries, Indianapolis, IN) were randomly assigned to one of two dietary groups and fed either basal (standard) chow (0.2% choline chloride) or choline supplemented chow (2.0% choline chloride) for 28–35 days. The diets were prepared by the Ralston Purina Company (Richmond, IN) and dietary analyses by gas chromatography [17] indicated that the standard diet contained 15.1 nmoles free choline/mg chow and the supplemented diet contained 141.3 nmoles free choline/mg chow. In addition, the lipid composition was determined to be 10.2% for both diets [14].

Sodium pentobarbital (Nembutal) was purchased from Abbott Laboratories (North Chicago, IL) and was used as supplied (50 mg/ml). For controls, animals received a buffered saline solution containing 40% propylene glycol and 10% ethanol.

Animals were maintained on a 12 hour light/dark cycle (6 a.m./6 p.m.) with ad lib access to food and water. Serum levels of both free and total choline were determined for both dietary groups. Blood was obtained by cardiac puncture and

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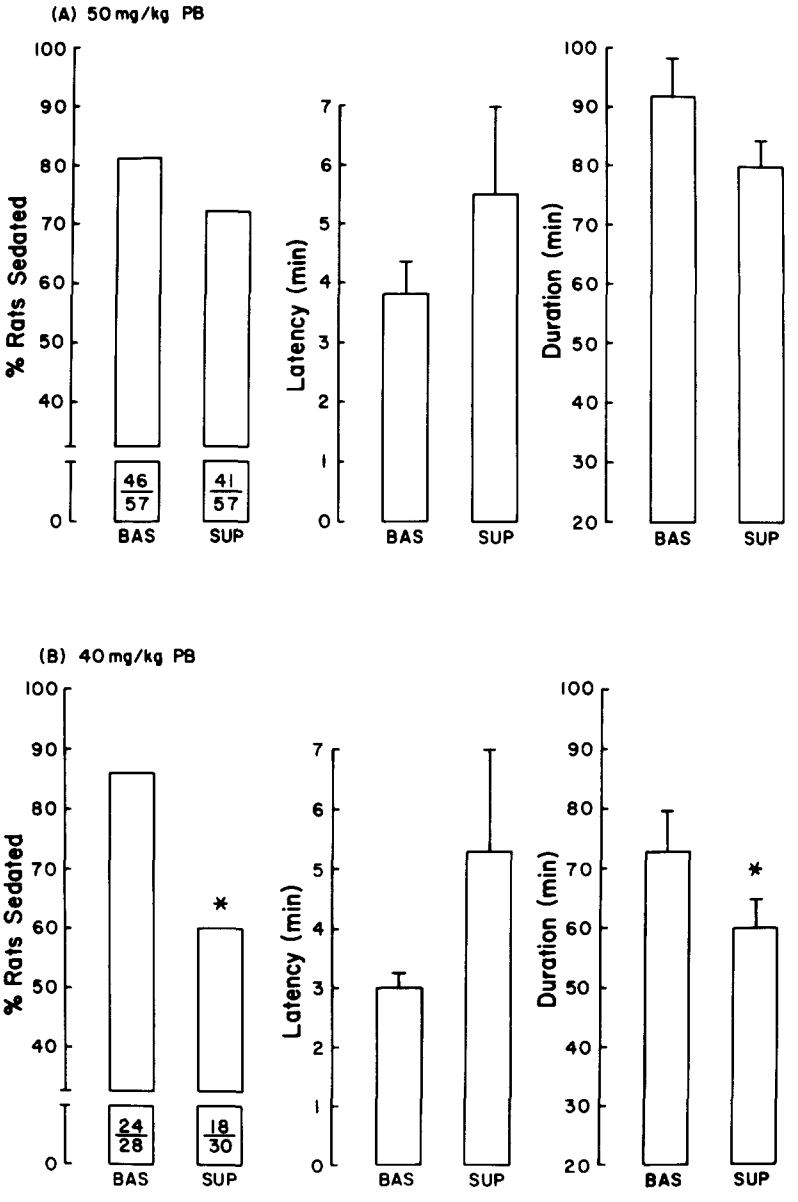

FIG. 1. Effects of chronic choline supplementation on the sedative/hypnotic actions of pentobarbital. Rats maintained on either basal (BAS) or choline supplemented (SUP) chow for 28–35 days received either 40 or 50 mg/kg pentobarbital (PB, IP) and the percent of animals exhibiting a loss of the righting reflex was determined. The number of animals sedated/the number of animals injected is noted on the graph. For those rats that became sedated, both the latency (time between injection and loss of reflex) and duration (time between loss of reflex and regaining reflex) were noted. Bars represent mean values±SEM. The asterisks denote significant ($p<0.05$) differences between groups as determined by the chi-square statistic (for sedation) or by the Student's t-test (for duration).

TABLE 1

EFFECTS OF DIETARY CHOLINE SUPPLEMENTATION ON THE HYPOTHERMIC EFFECT OF PENTOBARBITAL

Dose of PB	Dietary Regimen	Initial Temp (°C)	Maximal Δ Temp (−°C)	Time of Maximum (minutes)	Area Under Curve (°C × min)
50 mg/kg					
	Basal	36.1 ± 0.17	3.4 ± 0.23	85 ± 4.9	315 ± 28
	Supplemented	36.1 ± 0.14	3.2 ± 0.22	77 ± 5.9	285 ± 22
40 mg/kg					
	Basal	36.3 ± 0.16	2.9 ± 0.16	73 ± 6.2	252 ± 27
	Supplemented	36.2 ± 0.12	2.6 ± 0.13*	60 ± 3.8*	211 ± 25

Rats were maintained on the dietary regimens for 28–35 days and received either 40 or 50 mg/kg pentobarbital (PB) intraperitoneally. Each value is the mean of determinations from 11 (50 mg/kg) or 8 (40 mg/kg) rats/group ±S.E.M. Data were analyzed by the Student's *t*-test.

*Significantly less than corresponding basal group value, $p<0.05$.

TABLE 2

EFFECTS OF DIETARY CHOLINE SUPPLEMENTATION ON PENTOBARBITAL-INDUCED INCREASES IN ACH LEVELS

Diet	Striatum	Hippocampus	Cortex
		ACh (nmoles/g)	
		Vehicle-Injected	
Basal (n=11)	98.1 ± 4.2	28.8 ± 1.3	14.5 ± 0.4
Supplemented (n=11)	95.7 ± 4.4	30.2 ± 1.2	16.4 ± 0.9
		Pentobarbital-Injected (Sedated)	
Basal (n=16)	120 ± 3.3*	45.0 ± 1.3*	35.7 ± 1.6*
Supplemented (n=14)	125 ± 3.7*	45.8 ± 1.3*	35.7 ± 1.2*
		Pentobarbital-Injected (Non-Sedated)	
Basal (n=5)	105 ± 5.2	30.6 ± 2.9†	20.8 ± 1.8*†
Supplemented (n=9)	109 ± 4.7	32.3 ± 1.0†	18.8 ± 0.6†

Rats were maintained on the dietary regimens for 28–35 days and received either vehicle or 40 mg/kg pentobarbital (PB, IP) 30 minutes prior to killing by head-focused microwave irradiation. Levels of ACh were determined as described in the text. Each value is the mean ± S.E.M. The number of rats/group is in parentheses. Data were analyzed by ANOVA and significant differences determined by Newman-Keuls test.

*Significantly different from corresponding control (vehicle-injected) group value, $p<0.05$.

†Significantly different from corresponding PB-injected, sedated group values, $p<0.05$.

free choline was quantified by pyrolysis gas chromatography [17]. Total choline was determined using an oxidase/peroxidase system coupled to a dye reaction following phospholipase D hydrolysis of choline-containing lipids [23].

Behavioral Studies

For behavioral and metabolic studies, rats were kept in a sound- and temperature-controlled room. The effects of dietary choline supplementation on the sedative/hypnotic effects of pentobarbital were determined by monitoring the number of rats exhibiting a loss of the righting reflex. Animals were evaluated by a double-blind observer. In addition, for those animals that became sedated, the time between drug injection and the loss of the righting reflex (latency) as well as the time between loss of the reflex and regaining the reflex (duration) were noted. The statistical significance of differences between the 2 dietary groups was determined using the chi-square test (for the number of animals sedated) or the Student's *t*-test (for latency and duration) [24,31].

For studies determining the effects of choline supplementation on pentobarbital-induced hypothermia, the temperature of rats was measured at 10 minute intervals using a digital readout rectal probe (Electromedics, Inc., Denver, CO) for 40 minutes prior to and 150 minutes following the injection of pentobarbital. The change in temperature over time was plotted for each animal and the area bounded by each temperature curve was computed using the trapezoidal method [24,31].

The effects of choline supplementation on locomotor activity were determined by placing rats in a Plexiglas chamber (25×50 cm; 35 cm height) that had two photocells positioned to estimate horizontal movement. The number of photocell counts was automatically recorded per 10 minute interval. Statistical differences in activity for the first 30 minutes were tested using the Student's *t*-test [31].

Biochemical Studies

For the determination of ACh levels, rats received injections (IP) of either vehicle (0.1 ml/100 g body weight) or pentobarbital (40 mg/kg) and were killed 30 minutes after the injection by head-focused microwave irradiation [21]. The striatum, hippocampus, and midbrain cerebral cortex were dissected bilaterally, weighed, and homogenized (Polytron, Brinkman Inst., Westbury, NY) in acetonitrile containing propionylcholine iodide as an internal standard. ACh was quantified by pyrolysis gas chromatography [22].

To assess the possible effects of choline supplementation on peripheral metabolism, liver microsomes were prepared and protein content, levels of P-450, and the microsomal oxidation of benzphetamine and p-nitroanisole were determined [12]. Livers were removed, weighed, homogenized in phosphate buffer (50 mM, pH 7.4), and centrifuged at 9,000×g for 20 minutes at 4°C. Lipid was aspirated, and the supernatant was centrifuged at 100,000×g for 60 minutes at 4°C. The pellet was washed with 0.15 M KCl and recentrifuged at 100,000×g. The washed pellet was resuspended in 0.15 M KCl, and protein was determined using bovine serum albumin as standard [13]. Demethylation reactions were monitored on an SLM-Aminco DW-2C spectrophotometer (SLM Instruments, Inc., Urbana, IL).

The phosphorylation of 2-deoxyglucose (2-DG) was measured by a modification of the methods of Sokoloff *et al.* [19] and Crane *et al.* [5]. Rats were anesthetized with ether and the jugular vein was cannulated 24–36 hours prior to experimentation. Rats received injections (IP) of vehicle or pentobarbital (40 mg/kg) 10 minutes prior to the administration (IV) of 12 μCi 2-deoxy-1-^{3}H-glucose (2-DG, Amersham, Arlington Heights, IL). 2-DG was administered in a volume of 0.25 ml and the solution was immediately washed through the needle and the cannula with 0.2 ml saline. Animals were killed 45 minutes after the 2-DG injection by head-focused microwave irradiation and the brains were removed and chilled in ice-cold heptane. The striatum, hippocampus and midbrain cerebral cortex were dissected bilaterally and homogenized (teflon:glass) in water. Samples were centrifuged at 10,000×g for 30 minutes at 2°C. Aliquots of the supernatant were counted to monitor column recovery and pellets were used for protein determinations. One ml aliquots of the supernatants were applied to DEAE Sephadex columns and 2-DG was eluted with 4 ml water. The columns were washed with 2 ml water, and 2-DG-6-phosphate (2-DG-6-P) was eluted with 4×2 ml NaCl (1 M, pH 4.0 containing 0.1 M citric acid). Aliquots of the eluates were counted on a Beckman LS-3801 (Beckman Inst. Co., Houston, TX). Glucose was determined in both blood and brain tissue using a hexokinase/dehydrogenase coupled reaction. The reduction of NAD^+ was monitored spectrophotometrically at 340 nm on a Beckman DU-8 spectrophotometer. Results obtained from the biochemical studies were analyzed by analysis of variance (ANOVA) and significant differences determined by the Newman-Keuls test [7,31].

TABLE 3

EFFECTS OF DIETARY CHOLINE SUPPLEMENTATION ON INDICATORS OF LIVER MICROSOMAL METABOLISM

Parameter	Dietary Regimen	
	Basal	Supplemented
Protein Content (mg microsomal protein/g liver)	16.1 ± 0.3	16.3 ± 0.4
P-450 levels (nmoles/mg protein)	0.582 ± 0.04	0.600 ± 0.03
Benzphetamine demethylation (nmoles/mg protein/min)	2.79 ± 0.18	2.69 ± 0.91
P-nitroanisole demethylation (nmoles/mg protein/min)	1.56 ± 0.19	1.26 ± 0.26

Rats were maintained on the dietary regimens for 28–35 days. Liver microsomes were prepared and assayed as described in the text. Each value is the mean ± S.E.M. of determinations from 8 (protein content and P-450 levels) or 4 (demethylation reactions) animals.

RESULTS

Maintenance of rats on the choline supplemented diet for 28–35 days did not alter the general health of the animals as assessed by food and water intake and weight gain. Rats consumed 25±3 ml of water and 15±3 grams of chow per day, (mean±SEM, N=10) irrespective of dietary regimen. Similarly, the body weight gain for rats fed either the basal or supplemented diets was identical and was 7±2 grams per day. To ensure that the choline supplemented diet increased circulating levels of choline, the concentration of choline was measured in serum. Dietary supplementation increased the levels of

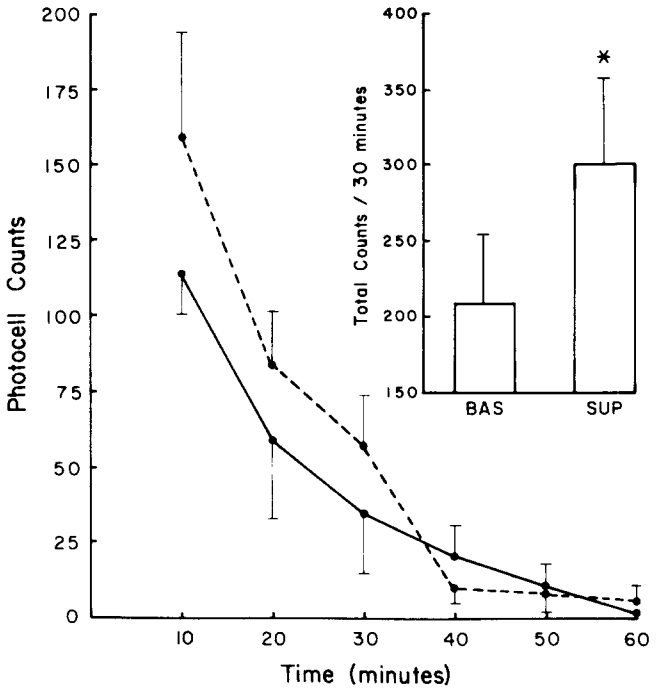

FIG. 2. Effects of chronic choline supplementation on locomotor activity. Rats were maintained on either basal (BAS, solid line) or choline supplemented (SUP, dotted line) chow for 28–35 days. Locomotor activity was determined by placing rats in a Plexiglas chamber (25×50 cm; 35 cm height) equipped with two photocells positioned to estimate horizontal movement, with photocell counts/10 minutes recorded automatically. Total activity for the first 30 minute period is shown in the insert. Values represent the mean±SEM of determinations from 5 rats/group. The asterisk denotes significant ($p<0.05$) differences between the two groups as determined by the Student's t-test.

free choline by 52%, from basal values of 14.7±1.1 μM to 22.4±1.1 μM. In addition, the concentration of the total circulating choline pool, which is composed primarily of lipid soluble choline esters, was also measured. The concentration of total choline in serum from rats fed the supplemented chow was 1.48±0.05 mM and was not different from that in serum from rats fed the basal chow (1.39±0.07 mM).

Initial experiments investigated the effects of dietary choline supplementation on the sedative/hypnotic effects of 50 mg/kg pentobarbital (Fig. 1A). This dose-produced sedation in 81% of basal rats while only 72% of rats in the supplemented group lost their righting reflex. In addition, for those rats that did exhibit sedation, the latency was increased by 40% and the duration of the response was decreased by 13% in the supplemented group. Since previous studies indicated that the effects of choline supplementation are dependent on the dose of the challenging agent [29], the experiment was repeated using lower doses of pentobarbital. Preliminary studies indicated that the administration of 40 mg/kg produced sedation in approximately the same number of rats as the higher dose, but the duration of the effect was shortened. Doses lower than 40 mg/kg gave highly variable results. Therefore, the effects of choline supplementation on the sedative/hypnotic effects of 40 mg/kg pentobarbital were determined (Fig. 1B). This dose of pentobarbital produced sedation in 86% of the rats in the control group, whereas the percent of rats sedated in the choline supplemented group was only 60% and was significantly ($p<0.05$) less than in the control group. In addition, for those rats that did exhibit sedation, the latency increased in the supplemented group (to 175% control), and the duration of the effect was significantly ($p<0.05$) reduced by 20%. Therefore, choline supplementation not only rendered rats less susceptible to the sedative effects of pentobarbital, but also attenuated the response in those animals that lost their righting reflex.

Since hypothermia occurs with anesthetic doses of the barbiturates, we determined whether choline supplementation affected the hypothermic response of pentobarbital in sedated rats (Table 1). Initial body temperatures did not differ between dietary groups. The hypothermic effect of 50 mg/kg pentobarbital was not altered significantly by choline supplementation, although values for all parameters measured were lower than in the control group. The hypothermia induced by the administration of 40 mg/kg pentobarbital was significantly attenuated by choline supplementation. Both the maximal change in temperature and the time to reach this maximum decreased by 10% and 18%, respectively, and the area of the temperature curve decreased by 16%.

Studies have shown that pentobarbital and other barbiturates increase the steady-state levels of ACh in brain by decreasing neurotransmitter release, effects that may be related to levels of consciousness (for review see [16]). Since the effects of choline supplementation have been postulated to be due to alterations in the metabolism of ACh in brain, experiments investigated whether the resistance of choline supplemented rats to the behavioral effects of pentobarbital was mediated through an attenuation of pentobarbital-induced alterations in ACh metabolism. The effects of choline supplementation on pentobarbital-induced increases in the levels of ACh are shown in Table 2. The striatum, hippocampus, and cerebral cortex were chosen for study because these 3 brain regions are rich in cholinergic innervation and have been shown to be responsive to manipulations by both choline supplementation [25, 28–30] and pentobarbital [16]. The levels of ACh in brains from vehicle-injected rats did not differ between the dietary groups, similar to previous results [26,27]. Furthermore, for those rats that exhibited sedation in response to pentobarbital, the induced increase in ACh levels was not altered by dietary choline supplementation; levels of ACh in striatum, hippocampus, and cortex increased by approximately 25%, 55%, and 125%, respectively, in both groups. In addition, choline supplementation did not alter pentobarbital-induced decreases in the rate of high affinity choline uptake into synaptosomes from sedated rats (data not shown). Similarly, when ACh levels were determined in rats that did not exhibit sedation in response to pentobarbital, no differences were apparent between the dietary groups. Thus, although the sedative/hypnotic and hypothermic effects of pentobarbital were attenuated by choline supplementation, no alterations in the cholinergic response to pentobarbital were observed.

Since results suggested that alterations in the cholinergic system did not mediate the effects of choline supplementation, studies were initiated to assess possible alterations in liver metabolism since dietary manipulations are generally associated with the induction of microsomal metabolism [12]. No differences were evident between dietary groups for any of the measurements (Table 3), suggesting that choline supplementation did not alter the microsomal metabolism of pentobarbital. Although we have not ruled out the possibility that the supplemented diet may have altered other pharmacodynamic and pharmacokinetic variables, it is unlikely

that a 10-fold increase in choline intake, without any other dietary alterations, would affect such parameters.

At the neuronal level, the barbiturates depress excitatory synaptic transmission at many central synapses and affect numerous other chemical processes. It is well documented that pentobarbital decreases glucose metabolism in brain [5,19], a process that is closely related to the functional activity of central neurons (for review see [18]). Since previous studies indicated that choline supplementation produces a behavioral hyperactivity [28], it was possible that the resistance of choline supplemented rats to the sedative effects of pentobarbital was mediated at the level of cerebral metabolism. To investigate this possibility, pentobarbital-induced metabolic depression was studied by investigating the phosphorylation of 2-DG in 3 brain regions (Table 4). Choline supplementation, by itself, increased the levels of 2-DG-6-P in the striatum and hippocampus by 14% and in the cortex by 27%. Since these increases could reflect alterations in either circulating or brain levels of glucose, the concentrations of glucose in both blood and brain were determined. No differences were noted between the dietary groups (blood levels of glucose were 1.37±0.05 mg/ml for the control group and 1.34±0.05 mg/ml for the supplemented group; brain concentrations were 0.72±0.05 mg glucose/g tissue for basal rats and 0.67±0.04 mg glucose/g tissue for supplemented animals; values are the means±SEM of determinations from 6 rats/group).

When the effects of pentobarbital were measured in brains from rats that exhibited sedation, the levels of 2-DG-6-P decreased by 41–56% in all regions from both dietary groups. Thus, choline supplementation did not modify the depression of glucose metabolism induced by pentobarbital in sedated rats. Similarly, when glucose metabolism was determined in brains from pentobarbital-injected rats that did not exhibit sedation, brain levels of 2-DG-6-P decreased to the same values in both dietary groups. The striatum and hippocampus exhibited a 20–30% inhibition. In the cortex, although the levels of 2-DG-6-P decreased to the same absolute value in both dietary groups, the percent decrease, relative to corresponding control (vehicle-injected) values was 31% for the basal group and 46% for the supplemented group, the latter reflecting the choline-induced increase in cortical metabolism. Thus, results from metabolic studies indicate that choline supplementation increases cerebral glucose metabolism, but does not affect responses to pentobarbital.

Since prior studies suggesting that choline induces a behavioral hyperactivity were investigated in rats maintained on diets different from those used in the present study [28], locomotor activity was monitored to determine whether the observed increase in cerebral metabolism was manifest at a behavioral level. When rats were placed in the behavioral apparatus following one month on the dietary regimens, rats maintained on the supplemented diet exhibited a 45% increase in activity during the first 30 minutes of monitoring (Fig. 2). Thereafter, no differences were apparent between the dietary groups. Thus, chronic supplementation with choline produced both a behavioral and neurochemical hyperactivity.

TABLE 4

EFFECTS OF DIETARY CHOLINE SUPPLEMENTATION ON PENTOBARBITAL-INDUCED DECREASES IN 2-DEOXYGLUCOSE METABOLISM

Diet	Striatum	Hippocampus	Cortex
	2-DG-6-P (fmoles/mg protein)		
	Vehicle-Injected		
Basal (n=11)	40.6 ± 2.6	28.6 ± 2.0	37.3 ± 1.9
Supplemented (n=11)	46.1 ± 2.6	32.6 ± 1.8	47.4 ± 3.3‡
	Pentobarbital-Injected (Sedated)		
Basal (n=11)	20.2 ± 1.7*	16.5 ± 1.0*	17.8 ± 0.9*
Supplemented (n=9)	21.1 ± 1.2*	19.1 ± 0.9*	20.7 ± 1.2*
	Pentobarbital-Injected (Non-Sedated)		
Basal (n=3)	29.0 ± 2.9*†	22.7 ± 1.7	25.9 ± 1.9*
Supplemented (n=6)	30.9 ± 1.4*†	23.5 ± 1.3*	25.7 ± 1.3*

Rats were maintained on the dietary regimens for 28–35 days and received either vehicle or 40 mg/kg pentobarbital (IP) 10 minutes prior to the injection (IV) of 12 μCi 2-deoxyglucose. The levels of 2-DG-6-P (2-deoxyglucose-6-phosphate) were determined as described in the text. Each value is the mean ± S.E.M. The number of rats/group is in parentheses. Data were analyzed by ANOVA and significant differences determined by Newman-Keuls test.

*Significantly different from corresponding control group values, $p<0.05$.

†Significantly different from corresponding sedated group values, $p<0.05$.

‡Significantly different from corresponding basal group values, $p<0.05$.

DISCUSSION

Results from the present studies indicate that chronic supplementation with choline renders rats resistant to the sedative/hypnotic effects of pentobarbital and attenuates the behavioral responses in those animals exhibiting a loss of the righting reflex. The effects of choline on brain have been postulated to be due to both direct as well as indirect actions. Although choline does possess agonist activity [2, 6, 8, 11, 15, 20], chronic supplementation with choline does not increase the levels of choline in brain [26–28]. In addition, choline supplementation does not alter the affinity or density of muscarinic receptors in brain, as assessed by quinuclidinyl benzilate and cis-dioxolane binding studies (unpublished observations). Thus, it is unlikely that the effects of choline supplementation reflect a direct receptor-mediated alteration.

In addition to a direct effect of choline on cholinergic neurons, it has been suggested that dietary choline supplementation enhances the synthesis and release of ACh [3]. However, chronic supplementation with choline does not increase the levels of ACh in brain ([26–28]; and see Table 2), and may decrease the turnover of ACh [1,10]. If the actions of choline supplementation to attenuate the behavioral effects of pentobarbital were mediated by alterations in the metabolism of ACh, one might expect a modification of the pentobarbital-induced increase in ACh levels or decrease in the rate of choline uptake. However, these actions of pentobarbital are not altered by choline supplementation,

suggesting that some other mechanism may be involved.

Studies elucidating the relationship between sedation and pentobarbital-induced decreases in glucose metabolism have shown that rats lose their righting reflex at doses of pentobarbital that decrease the rate of glucose utilization in brain by 44–55%, while lower doses only lead to sluggish and staggering behavior [5]. These observations are supported by results from the present study indicating a 41–56% decrease in 2-DG-6-P levels in all brain regions examined from sedated rats, irrespective of dietary group. Furthermore, in non-sedated rats, the levels of 2-DG-6-P decreased to the same absolute values and were below that necessary to induce a loss of the righting reflex.

Although results indicate that choline supplementation does not alter pentobarbital-induced decreases in cerebral glucose metabolism, choline supplementation, by itself, increased metabolism. It is possible that this enhanced level of functional neuronal activity mediates both the increased locomotor activity and the resistance of these animals to the sedative/hypnotic effects of pentobarbital. Since the present study investigated only those brain regions known to be densely innervated by cholinergic neurons, it is possible that other areas, especially those associated with arousal mechanisms, may exhibit both an enhanced metabolic activity and a resistance to the depression induced by pentobarbital. Lastly, although the specific cellular mechanisms underlying the effects of choline supplementation on cerebral glucose metabolism have not been elucidated, they may involve choline-induced alterations in phospholipid metabolism with consequent membrane modifications [26,27].

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