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BRIEF COMMUNICATION

Choline Administration: Lack of Effect on Plasma Catecholamines in Rats[1]

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McCARTY, R. *Choline administration: Lack of effect on plasma catecholamines in rats.* PHARMACOL BIOCHEM BEHAV 18(4)651–653, 1983.—Choline chloride (35 or 70 mg/kg, IP) or saline was administered daily for 3 consecutive days to adult male Sprague-Dawley rats. Before and 30, 60 and 120 minutes after the third injection of choline chloride or saline, blood samples were collected from a chronic tail artery catheter and later analyzed for levels of norepinephrine (NE) and epinephrine (EPI). Plasma levels of both catecholamines did not differ between choline- and saline-injected rats at either of the four sampling points. When insulin (10 IU/kg, SC) was administered to stimulate the sympathetic-adrenal medullary system reflexly, plasma levels of NE and EPI increased significantly above basal values but were similar for choline- and saline-injected rats. These findings do not support a role for choline availability in the regulation of catecholamine secretion from the adrenal medulla.

Plasma catecholamines Norepinephrine Epinephrine Choline chloride Sympathetic nervous system Adrenal medulla

CONSIDERABLE evidence has been presented to indicate a role for choline availability in the regulation of acetylcholine synthesis and release in the central nervous system and in the adrenal medulla (for review, see [11]). Chronic administration of choline chloride or lecithin to rats results in a rapid elevation of choline and acetylcholine levels in the sympathetic preganglionic terminals in the adrenal medulla [4,10]. These changes are followed somewhat later by an induction of adrenal tyrosine hydroxylase activity that is blocked by denervation of the adrenals or by concurrent administration of the protein synthesis inhibitor, cycloheximide [9,10]. The effects of choline chloride on the synthesis and release of adrenal catecholamines are thought to result from an enhanced release of acetylcholine from splanchnic nerve terminals with each depolarization [7,9].

In the present study, this suggestion was tested by examining the effects of choline administration on basal and stress-induced increases in plasma norepinephrine (NE) and epinephrine (EPI) in rats. If choline administration does stimulate an increase in acetylcholine synthesis and release, then it follows that this compound should increase plasma levels of EPI and to a lesser extent NE under resting conditions and especially after treatments which stimulate the sympathetic-adrenal medullary system.

METHOD

Adult male Sprague-Dawley rats (Dominion Laboratories, Dublin, VA), weighing 325–450 g, were isolated in clear plastic cages (25×25×15 cm) for at least 1 week prior to use. Rats were provided with ad lib supplies of water and laboratory chow and room lights were on from 0700–1900 hours.

For the first experiment, rats were weighed and injected with choline chloride (Sigma, 35 or 70 mg/kg, IP) or an equal volume of saline (1.0 ml/kg, IP). At least 1 hour after the injection, rats were anesthetized with pentobarbital (40 mg/kg, IP) and a PE50 catheter was inserted into the ventral caudal artery and secured with 2 sutures. The tubing was then run under the tail sheath and skin to exit at the back of the neck [1]. After recovery from anesthesia, rats were returned to their home cages and the catheters were allowed to hang freely from the tops of the cages. To maintain patency, catheters were flushed in the early morning and late afternoon with 0.5 ml of the heparinized saline (300 IU/ml).

On the morning after surgery, rats received a second injection of choline chloride or saline as described above. Two days after surgery, blood samples (0.5 ml) were collected from each rat between 1000–1130 hours with a minimum of disturbance. Next, rats received a third injection of the appropriate dose of choline chloride or saline and blood samples were collected 30, 60 and 120 minutes later.

In a second experiment, rats were weighed and injected with choline chloride (70 mg/kg, IP) or an equal volume of saline (1.0 ml/kg, IP) and then surgically prepared with chronic tail artery cannulae as described above. Rats then received injections of choline chloride or saline on each of the two mornings after surgery. One hour after the third such injection, basal blood samples were collected from rats

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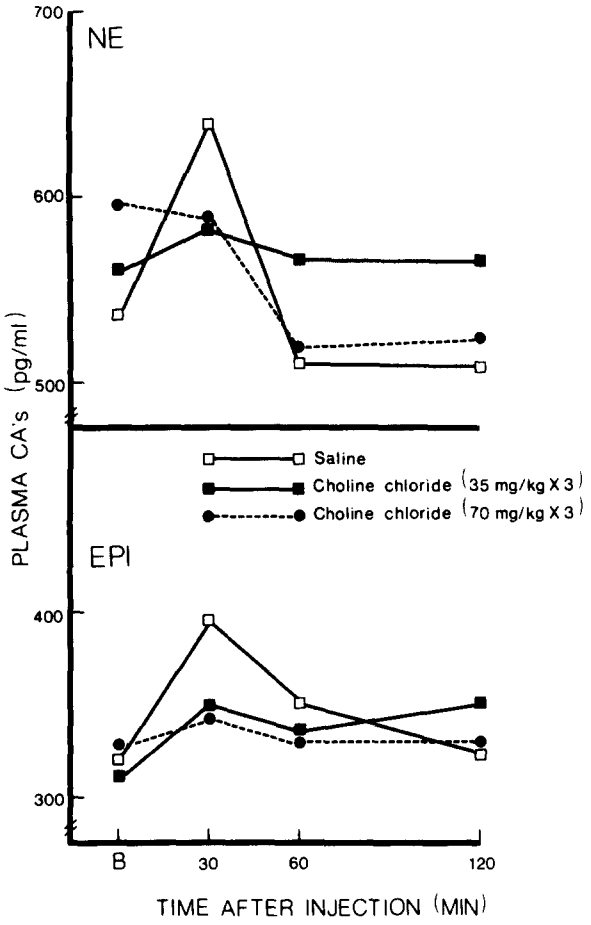

FIG. 1. Effects of choline chloride on plasma levels of norepinephrine (NE) and epinephrine (EPI) in undisturbed rats. Choline chloride (35 or 70 mg/kg, IP) was administered daily for 3 consecutive days and arterial blood samples were collected immediately before (B) and 30, 60 and 120 minutes after the third injection. Control animals received injections of saline. Values are means for groups of 5 rats and SEMs were 10–20% of the means. Note the discontinuous ordinate scale.

of the two treatment groups. Then, each rat received an injection of insulin (Lilly, 10 IU/kg, SC) and blood samples were obtained at 30, 60, 120 and 180 minutes after the insulin injection.

Blood samples were collected into iced 10×75 mm glass tubes, centrifuged at 4000 ×g for 10 minutes at 4°C and 200 μl of cell-free plasma were removed and frozen at −20°C. Within 4 weeks, samples were assayed for content of norepinephrine and epinephrine by a radioenzymatic-thin layer chromatographic method [6].

Data for each experiment are expressed as mean values±SEM for groups of 5–8 rats. Results were analyzed by a one-way ANOVA or by student's *t*-test as appropriate.

RESULTS

Administration of choline chloride (35 or 70 mg/kg) to rats for two days had no effect on basal plasma levels of norepinephrine and epinephrine when compared to saline-injected controls, p's>0.30. Similarly, plasma levels of both catecholamines were unaffected for up to 2 hours after a third injection of choline chloride when compared to saline controls, p's>0.20 (Fig. 1).

TABLE 1

EFFECTS OF INSULIN ON PLASMA LEVELS OF NOREPINEPHRINE (NE) AND EPINEPHRINE (EPI) IN SALINE AND CHOLINE CHLORIDE TREATED RATS

	Saline	Choline
Plasma NE (pg/ml)		
Basal	776 ± 76	727 ± 33
Peak response	1101 ± 88*	1093 ± 73*
Plasma EPI (pg/ml)		
Basal	459 ± 21	400 ± 20
Peak response	1552 ± 271†	1626 ± 288†

Saline or choline chloride (70 mg/kg, IP) was injected daily for 3 consecutive days. Insulin (10 I.U./kg) was injected 1 hour after the third injection. Peak response refers to the highest value of each catecholamine in blood samples collected 30, 60,120 and 180 minutes after the administration of insulin.

Values are means ± SEM for groups of 6 rats.

*p<0.05 †p<0.01 compared to basal values (two-tailed t-test).

Consistent with the findings described above, plasma levels of norepinephrine and epinephrine were similar in rats that received choline chloride (70 mg/kg daily for 3 days) or saline. Administration of insulin (10 IU/kg) resulted in a significant increase above basal values in plasma levels of epinephrine, p's<0.01, and to a lesser extent norepinephrine, p's<0.05, in saline- and choline chloride treated rats. As the insulin-induced activation of the sympathetic-adrenal medullary system persisted for up to 3 hours, peak responses were determined for each rat by noting the highest level of plasma norepinephrine and plasma epinephrine across the 4 sampling points from 30 to 180 minutes after administration of insulin. The peak plasma norepinephrine and epinephrine responses after injection of insulin were approximately 145% and 370% of basal values, respectively. However, the plasma catecholamine response to insulin-induced hypoglycemia did not differ for choline chloride versus control rats (Table 1).

DISCUSSION

The present results indicate that chronic intraperitoneal administration of choline chloride to rats had little or no effect on basal or stress-induced increases in plasma levels of norepinephrine and epinephrine. These findings do not agree with the studies of Wurtman and his co-workers in which choline supplementation via intraperitoneal, intragastric or dietary routes has been reported to increase the synthesis and concentration of acetylcholine in central cholinergic neurons and in preganglionic nerve terminals in the adrenal medulla [11].

Increased intraneuronal acetylcholine concentrations following precursor loading (i.e., with choline chloride or lecithin) are thought to result in an increase in the amount of acetylcholine released per depolarization [11]. In the periphery, this diet-induced alteration in the release of acetylcholine results in an induction in the activity, and presumably the amount, of the catecholamine biosynthetic enzyme, tyrosine hydroxylase (TH) in adrenal medullary

chromaffin cells. Oral administration of choline chloride to rats also potentiated the induction of TH in the adrenal medulla following a variety of treatments known to increase the firing rate of the splanchnic nerves [10]. Finally, chronic administration of choline chloride to rats resulted in a significant increase in the levels of epinephrine in the urine [7].

A consideration of the methods to assess the secretory activity of the adrenal medulla may provide some insight into the differences between the present study and those of Wurtman and his co-workers. Recent studies have shown that the activity of TH in the adrenal medulla provides little information regarding the release of catecholamines from this gland [8]. Thus, a variety of conditions which induce TH in the adrenal medulla fail to increase or may actually decrease the secretion of epinephrine into blood during stressful conditions. In addition, the induction of TH in the adrenal medulla following chronic treatment of animals with choline chloride may result from a direct action of choline on nicotinic receptors of the chromaffin cells and not be indicative of an alteration in acetylcholine synthesis or release from preganglionic nerve terminals.

The adrenal medulla is virtually the sole source of epinephrine in the blood. Following its release from adrenal medullary chromaffin cells, epinephrine enters the circulation and has a half-life in rats of approximately 70 seconds. Catecholamines, including epinephrine, are cleared from plasma by several processes, including uptake into neuronal ($uptake_1$) and non-neuronal cells ($uptake_2$), sulfate conjugation, and O-methylation within liver, kidney and other peripheral tissues. A very small proportion (less than 5%) of the total amount of epinephrine released into blood enters the urine unmetabolized. Thus, a very small increase in the percentage of epinephrine which enters the urine unmetabolized could result in a striking increase in the amount of epinephrine excreted over a 24 hour period without any change in the amount of epinephrine released from the adrenal medulla during that same time [5].

In contrast to these indirect methods for assessing adrenal medullary activity (TH activity, urinary epinephrine levels), I have measured plasma levels of epinephrine and norepinephrine in rats treated for 3 days with choline chloride. Plasma levels of epinephrine have been shown to provide a sensitive and reliable index of adrenal medullary discharge [5]. In the present study, plasma epinephrine was not increased in choline-treated rats under resting conditions or following the administration of insulin, which results in a prolonged increase in adrenal medullary discharge. Similarly, other investigators have been unable to increase acetylcholine synthesis and release in brain following choline loading [2,3].

In summary, the present findings do not support the suggestion that choline administration increases the secretion of catecholamines from the adrenal medulla.

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