

Peripheral administration of CDP-choline and its cholinergic metabolites increases serum insulin: Muscarinic and nicotinic acetylcholine receptors are both involved in their actions

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Abstract

The present study was designed to test the effects of CDP-choline and its metabolites on serum insulin concentrations in rats and to investigate the involvements of cholinergic and adrenergic receptors in the effect. Intraperitoneal (i.p.) administration of CDP-choline (200–600 $\mu\text{mol/kg}$) increased serum insulin in a dose- and time-related manner. Equivalent doses (200–600 $\mu\text{mol/kg}$; i.p.) of phosphocholine or choline also increased serum insulin dose-dependently. Serum-free choline concentrations increased several-fold following i.p. administration of CDP-choline, phosphocholine or choline itself. In contrast, equivalent doses of cytidine monophosphate and cytidine failed to alter serum insulin concentrations. The increases in serum insulin induced by i.p. 600 $\mu\text{mol/kg}$ of CDP-choline, phosphocholine or choline were abolished by pretreatment with the ganglionic nicotinic acetylcholine receptor antagonist hexamethonium (15 mg/kg; i.p.), or by the muscarinic receptor antagonist atropine methylnitrate (2 mg/kg; i.p.). Pretreatment with prazosin (0.5 mg/kg; i.p.), an α_1 -adrenoceptor antagonist, or yohimbine (5 mg/kg; i.p.), an α_2 -adrenoceptor antagonist, enhanced slightly the increases in serum insulin in response to 600 $\mu\text{mol/kg}$ of CDP-choline, phosphocholine and choline. Serum insulin also increased following central administration of choline; the effect was blocked by intracerebroventricularly injected atropine, mecamylamine or hemicholinium-3 (HC-3). It is concluded that CDP-choline or its cholinergic metabolites phosphocholine and choline increases circulating insulin concentrations by increasing muscarinic and nicotinic cholinergic neurotransmission in the insulin secreting β -cells.

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CDP-choline is an endogenous intermediate in the biosynthesis of phosphatidylcholine. Administration of CDP-choline has been shown to exhibit beneficial effects in various neurodegenerative as well as ischemia- and stroke-related conditions both experimentally and clinically [1,19]. CDP-choline is also a prescription drug in several European countries and in Japan. When administered, CDP-choline is metabolized completely to form choline and cytidine/uridine which results in elevations in plasma and tissue levels of these metabolites [14,23] that are then utilized for the synthesis of phospholipids and acetylcholine [14,22,23]. CDP-choline treatment likewise increases cholinergic neurotransmission in central nervous sys-

tem which leads to neuroendocrine effects of cholinergic nature [5,16].

In the present study, we have investigated the effects of intraperitoneal (i.p.) administration of CDP-choline and its metabolites on serum insulin concentrations. It is known that both the peripheral and the central parts of parasympathetic branch of autonomic nervous system have important roles in the regulation of insulin release from endocrine pancreas [2,7]. Recent studies from our laboratory have shown that i.p. administration of choline, a precursor of the neurotransmitter acetylcholine, stimulates insulin release and elevates circulating insulin levels [11].

Female Wistar rats (Experimental Animals Breeding and Research Center, Uludag University, School of Medicine, Bursa, Turkey) weighing 250–275 g were used in all experiments. Four rats were housed in hanging cages with free access to food and

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water. The colony room was maintained at 20–24 °C with a 12 h light–dark cycle (light on 08 00–20 00 h).

The surgical and experimental protocols were approved by the Animals Care and Use Committee of Uludag University. All experiments were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. All efforts were made to minimize the number of animals used and their suffering.

For repeated blood withdrawal in the time–course studies, a PE 50 cannula was inserted into the left carotid artery as described previously [11]. For intracerebroventricular (i.c.v.) injections, a burr hole was drilled through the skull as described previously [3].

In the time–course studies, a blood sample (0.150 ml) was withdrawn from the arterial catheter immediately before (0) and 10, 20, 30, 45 and 60 min after i.p. treatments for serum insulin measurements. In all other studies, rats were killed by rapid decapitation 10 min after i.p. treatments and trunk blood was collected for serum insulin measurements. Blood samples were kept on ice and serum was obtained by centrifugation (1500 × *g* for 10 min) at 4 °C.

Serum insulin was determined by radioimmunoassay using a commercially available assay kit specific for rat insulin (Cat # RPA 547; Amersham Pharmacia Biotech, Buckinghamshire, England), as described previously [11]. The radioimmunoassay kit reliably detected 10–5000 pg of rat insulin per assay tube and 25–50 μl of serum used for each analysis. The intra- and inter-assay coefficients of variability for the rat insulin assay were about 13% and 6%, respectively. The cross-reactivity of the rat insulin antibody with rat pancreatic polypeptide, rat pancreastatin, rat amylin, somatostatin or C-peptide was less than 0.01%.

Free choline was assayed in 5 μl serum radioenzymatically, as described previously [11]. Serum glucose concentrations were determined in 5 μl of serum with the glucose oxidase method using a commercially available assay kit (Biotrol, France), per manufacturer instructions.

The following drugs were used: cytidine 5′-diphosphocholine sodium (CDP-choline) choline chloride, phosphocholine chloride, cytidine, cytidine monophosphate, atropine methylnitrate, atropine sulfate, mecamlamine hydrochloride, hexamethonium hydrochloride, hemicholinium-3 bromide (HC-3), prazosin hydrochloride, yohimbine hydrochloride, propranolol hydrochloride (Sigma Chemical Co., St. Louis, MO, USA).

Data were analyzed using one- or two-way repeated measures analyses of variance (ANOVA) followed by *post hoc* Tukey test. Values of *P* less than 0.05 were considered to be significant. Data are presented as the mean ± S.E.M.

Baseline serum insulin concentration prior to i.p. saline or CDP-choline injection was 2.0 ± 0.1 ng/ml (*n* = 6) or 2.1 ± 0.2 ng/ml (*n* = 6), respectively. As seen in Fig. 1, i.p. administration of CDP-choline increased serum insulin in a time—($F(5, 30) = 7.61, P < 0.001$) and dose-dependent ($F(3, 20) = 4.21, P < 0.01$) manner (Fig. 1A and B). Intraperitoneal administration of equivalent doses of choline (200–600 μmol/kg) also increased serum insulin in a

time—($F(5, 30) = 45.67, P < 0.001$) and dose-related ($F(3, 20) = 21.48, P < 0.001$) manner (Fig. 1C and D). Phosphocholine, a cholinergic metabolite of CDP-choline, also increased ($F(3, 28) = 6.00, P < 0.01$) serum insulin concentrations (Fig. 1E). Serum-free choline concentrations were 10.8 ± 0.8 μmol/l in saline-treated control rats; they increased to 65 ± 12, 147 ± 21 and 206 ± 9 μmol/l; 76 ± 13, 139 ± 16 and 198 ± 34 μmol/l; or 108 ± 18, 155 ± 18 and 255 ± 33 μmol/l at 10 min after 200, 400 and 600 μmol/kg of CDP-choline; phosphocholine; or choline, respectively. There was a highly significant and positive ($r = 0.946, P < 0.001$) correlation between serum insulin and free choline levels.

In contrast, neither cytidine (Fig. 1F) nor cytidine monophosphate (CMP; data not shown), the pyrimidineric metabolites of CDP-choline, altered serum insulin levels at equivalent doses (200–600 μmol/kg).

Pretreatment of rats with a nonselective peripheral muscarinic acetylcholine receptor antagonist, atropine methylnitrate (2 mg/kg; i.p.) or a ganglionic nicotinic acetylcholine receptor antagonist hexamethonium hydrochloride (15 mg/kg; i.p.) prevented the increases in serum insulin induced by CDP-choline, phosphocholine or choline (each 600 μmol/kg) (Table 1).

To determine whether the blockade of peripheral adrenoceptors affected serum insulin response to CDP-choline, phosphocholine and choline, rats were pretreated with saline (1 mg/kg); prazosin (0.5 mg/kg), a selective α₁-adrenoceptor antagonist; yohimbine (5 mg/kg), a selective α₂-adrenoceptor antagonist; or propranolol (2 mg/kg), a nonselective β-adrenoceptor antagonist, 15 min prior to i.p. administration of

Table 1
Effects of acetylcholine receptor antagonists on the increases in serum insulin elicited by CDP-choline, phosphocholine and choline

Pretreatment + treatment	<i>N</i>	Insulin (ng/ml)
Saline		
Saline + saline	6	2.8 ± 0.3
Saline + CDP-choline	6	4.1 ± 0.3*
Saline + phosphocholine	6	5.2 ± 0.4*
Saline + choline	6	6.5 ± 0.9*
Atropine methylnitrate		
Atropine methylnitrate + saline	6	2.7 ± 0.3
Atropine methylnitrate + CDP-choline	6	3.0 ± 0.5**
Atropine methylnitrate + phosphocholine	6	2.6 ± 0.3**
Atropine methylnitrate + choline	6	2.8 ± 0.23**
Hexamethonium		
Hexamethonium + saline	6	3.3 ± 0.3
Hexamethonium + CDP-choline	6	3.1 ± 0.5**
Hexamethonium + phosphocholine	6	2.5 ± 0.3**
Hexamethonium + choline	6	2.6 ± 0.2**

Rats were pretreated i.p. with saline (1 ml/kg), atropine methylnitrate (2 mg/kg) or hexamethonium (15 mg/kg) 15 min prior to i.p. administration of saline (1 ml/kg), CDP-choline, phosphocholine, or choline (each 600 μmol/kg). Animals were sacrificed 10 min after the second i.p. injection and blood samples were collected for serum insulin measurements. Data are expressed as the mean ± S.E.M. Data were analyzed by two-way ANOVA followed by Tukey's test.

* $P < 0.001$ compared with the values for "saline + saline".

** $P < 0.01$ compared with the respective values for "saline-pretreated" groups.

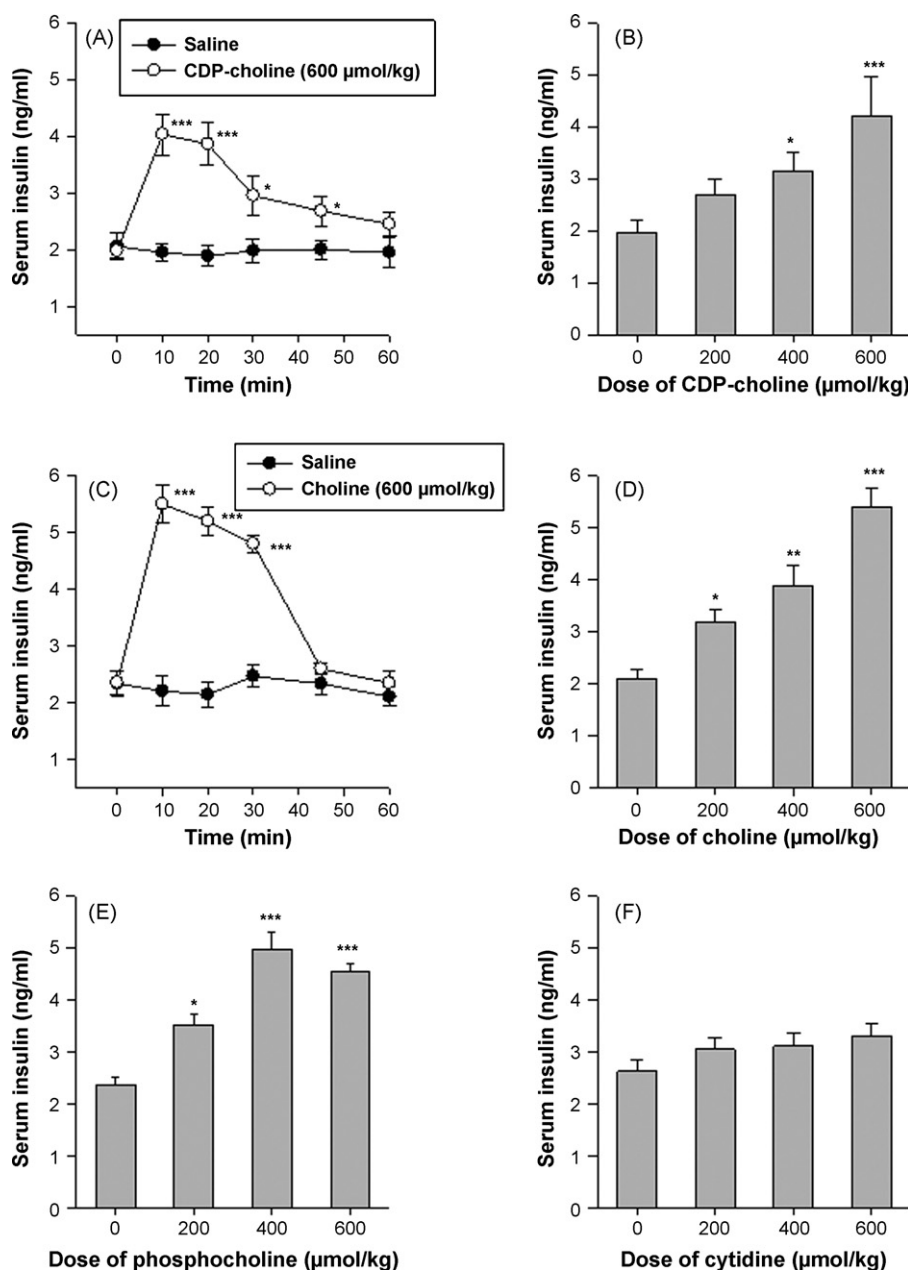


Fig. 1. Effects of CDP-choline and its metabolites on serum insulin concentrations. *Time-course study*: Rats were injected i.p. with either saline (1 ml/kg) or 600 μmol/kg of CDP-choline (A) or choline (C). Blood samples (1 ml) were collected immediately before (0 min), and 10, 20, 30, 45 and 60 min after each treatment through the catheter inserted into the left carotid artery and were analyzed for insulin. Each point represents the mean ± S.E.M. of six measurements. Data were analyzed using two-way ANOVA followed by Tukey's test. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ compared with the same time point from saline-treated controls. *Dose-course study*: Rats were injected i.p. with either saline (1 ml/kg), CDP-choline (B), choline (D), phosphocholine (E) or cytidine (F) (each 200–600 μmol/kg). Rats were sacrificed at 10 min after the treatment and blood samples were assayed for serum insulin. Each point represents the mean ± S.E.M. of six measurements. Data were analyzed using one-way ANOVA followed by Tukey's test. * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ compared with the same time point from saline-treated controls.

CDP-choline, phosphocholine or choline (each 600 μmol/kg). Pretreatment with propranolol did not alter serum insulin and glucose responses, while pretreatment with prazosin enhanced slightly the increase in serum insulin and glucose concentrations in response to CDP-choline, phosphocholine or choline (Table 2). Pretreatment with yohimbine also enhanced the increase in serum insulin, while it blocked the increase in serum glucose in response to CDP-choline, phosphocholine or choline (Table 2).

To determine whether central administration of choline affected serum insulin, rats were injected i.c.v. with choline (1.5 μmol). Serum insulin concentrations increased significantly ($P < 0.001$) to 4.2 ± 0.3 ng/ml ($n = 9$) 15 min following i.c.v. injection of choline compared with those (2.0 ± 0.1 ng/ml) in i.c.v. saline-treated rats. Pretreatment with mecamylamine (50 μg; i.c.v.), an antagonist of neuronal nicotinic acetylcholine receptors, or atropine (10 μg; i.c.v.), a muscarinic cholinergic antagonist, attenuated serum insulin response to i.c.v. choline

Table 2
Effects of adrenoceptor antagonists on the increases in serum insulin and glucose elicited by CDP-choline, phosphocholine and choline

Pretreatment/treatment	N	Insulin (ng/ml)	Glucose (mg/dl)
Saline			
Saline + saline	6	2.2 ± 0.2	132 ± 4
Saline + CDP-choline	6	4.1 ± 0.4*	155 ± 6*
Saline + phosphocholine	6	5.5 ± 0.5*	168 ± 6*
Saline + choline	6	6.0 ± 0.5*	187 ± 11*
Propranolol			
Propranolol + saline	6	1.7 ± 0.3	131 ± 6
Propranolol + CDP-choline	6	4.8 ± 0.5*	161 ± 5*
Propranolol + phosphocholine	6	5.6 ± 0.5*	176 ± 6*
Propranolol + choline	6	5.8 ± 0.7*	201 ± 8*
Prazosin			
Prazosin + saline	6	2.2 ± 0.2	179 ± 7
Prazosin + CDP-choline	6	6.1 ± 0.5*,**	215 ± 6*,**
Prazosin + phosphocholine	6	6.2 ± 0.4*,**	240 ± 11*,**
Prazosin + choline	6	7.8 ± 0.6*,**	233 ± 13*,**
Yohimbine			
Yohimbine + saline	6	2.4 ± 0.2	133 ± 4
Yohimbine + CDP-choline	6	6.6 ± 0.7*,**	135 ± 4**
Yohimbine + phosphocholine	6	7.7 ± 0.7*,**	132 ± 5**
Yohimbine + choline	6	8.2 ± 0.6*,**	134 ± 4**

Rats were pretreated i.p. with saline (1 ml/kg), propranolol (2 mg/kg), prazosin (0.5 mg/kg) or yohimbine (5 mg/kg) 15 min prior to i.p. administration of saline (1 ml/kg), CDP-choline, phosphocholine, or choline (each 600 µmol/kg). Animals were sacrificed 10 min after the second i.p. injection and blood samples were collected for serum insulin measurements. Data are expressed as the mean ± S.E.M. Data were analyzed by two-way ANOVA followed by Tukey's test.

* $P < 0.05$ – 0.001 compared with the values for the respective control "saline-treated".

** $P < 0.05$ compared with the respective values from "saline-pretreated" groups.

(Table 3). Pretreatment with HC-3 (20 µg; i.c.v.), a neuronal choline-uptake inhibitor, prevented the increase in serum insulin in response to i.c.v. choline (Table 3).

These data show that i.p. administration of CDP-choline or its cholinergic metabolite choline increases serum insulin concentrations in a dose- and time-related manner. Phosphocholine also increases serum insulin. Blockade of peripheral muscarinic and nicotinic acetylcholine receptors prevents the increase in serum insulin elicited by CDP-choline, phosphocholine and choline.

The increases in serum insulin in response to CDP-choline, phosphocholine and choline were associated with several-fold elevations in serum-free choline concentrations which varied between 65 ± 12 and 255 ± 33 µmol/l 10 min after i.p. administration of these compounds at 200–600 µmol/kg dose range. Previous studies have clearly shown that choline at a concentration within 10–130 µM range, which is attainable in the circulation following administration of 200–600 µmol/kg of CDP-choline, phosphocholine, or choline itself, enhances acetylcholine synthesis and release in the isolated pancreatic tissue which contains both pre- and post-ganglionic cholinergic parasympathetic neurons [11]. Taken together, it is reasonable to assume that the observed increase in serum insulin concentrations

following i.p. administration of choline, phosphocholine or CDP-choline mainly, but not necessarily solely, results from the increased cholinergic transmission in insulin secreting pancreatic β-cells due to precursor action of elevated serum choline. Failure of CMP or cytidine, administered in equivalent doses, to affect serum insulin suggests that, at least within the dose range used in this study, these pyrimidineric metabolites are not likely involved in the observed serum insulin response to CDP-choline.

Our finding that the increases in serum insulin concentrations induced by CDP-choline, phosphocholine or choline were blocked by both the muscarinic acetylcholine receptor antagonist atropine and the ganglionic nicotinic acetylcholine receptor antagonist hexamethonium shows that the effects of these choline compounds are mediated by both muscarinic and nicotinic acetylcholine receptors. These findings are in good agreement with previous studies demonstrating the involvement of both muscarinic and nicotinic acetylcholine receptors in the regulation of insulin release by cholinergic agonists [4,6,12,15] and by choline itself [11]. Although we did not test in the present study, we previously showed that M₁ and M₃ muscarinic acetylcholine receptor subtypes were mainly involved in the increase in serum insulin induced by i.p. administration of choline [11].

We have previously shown that choline [10] or CDP-choline [9,18] activates sympatho-adrenal systems, increases plasma catecholamines [9,10,18], and induces hyperglycemia [9,10]. The release of insulin from the pancreas is known to be inhibited by catecholamines [13,16,17] and stimulated by direct action of glucose on β-cells; this glucose-induced insulin release is enhanced by choline [11] and cholinergic agonist [4]. In the present study, we demonstrate that serum insulin responses to CDP-choline, phosphocholine and choline are not influenced by β-adrenoceptor blockade, but are enhanced slightly by both α₁-adrenoceptor and α₂-adrenoceptor blockade. In accordance with previous study [9], our data also show that α₂-adrenoceptor blockade by yohimbine abolishes completely the hyperglycemic responses to CDP-choline and its

Table 3
Effects of atropine, mecamylamine or HC-3 on the increases in serum insulin elicited by i.c.v. choline

Pretreatment + treatment	N	Insulin (ng/ml)
Saline + saline	7	2.2 ± 0.2
Saline + choline	7	4.3 ± 0.3*
Atropine + saline	7	2.1 ± 0.3
Atropine + choline	7	2.7 ± 0.2**
Mecamylamine + saline	7	2.4 ± 0.2
Mecamylamine + choline	7	3.1 ± 0.2**
HC-3 + saline	7	2.4 ± 0.2
HC-3 + choline	7	2.8 ± 0.4**

Rats were pretreated i.c.v. with saline (10 µl), atropine (10 µg), mecamylamine (50 µg) or HC-3 (20 µg) 15 min prior to i.c.v. administration of saline (10 µl) or choline (1.5 µmol). Animals were sacrificed 10 min after the second i.c.v. injection and blood samples were collected for serum insulin measurements. Data were analyzed by two-way ANOVA followed by Tukey's test.

* $P < 0.001$ compared with the values from "saline + saline" group.

** $P < 0.05$ compared with the values from "saline + choline" group.

cholinergic metabolites (Table 2). This observation suggests that the rise in insulin is not a simple response to increased serum glucose. Furthermore, since catecholamine-induced inhibition of insulin secretion involves α_2 -adrenoceptors [13,16,17], the observed enhancement in serum insulin response in α_2 -adrenoceptors-blocked rats suggests that α_2 -adrenoceptor activation counteracts parasympathetic activation of insulin secretion. Contrary to α_2 -adrenoceptor blockade, the enhanced serum insulin response to CDP-choline and its cholinergic metabolites in α_1 -adrenoceptors-blocked rats was accompanied by greater hyperglycemia; the effect may be resulted, in part, with further stimulation of insulin release by higher serum glucose concentrations as shown previously by choline [11] and cholinergic agonists [4]. Hyperglycemic response to choline [9,10] and choline compounds [9] involves adrenal medulla activation and mediates α_2 -adrenoceptors [9]. The observed greater hyperglycemia in α_1 -adrenoceptors-blocked rats could be explained by the fact that effect of choline on cholinergic neurotransmission in the sympatho-adrenal system enhanced by treatments (i.e., α -adrenoreceptor blockade, chemical sympathectomy) that are known to increase the firing rates of preganglionic cholinergic nerves [21]. Taken together, it is reasonable to conclude that simultaneous activation of the sympatho-adrenal system alters parasympathetically mediated insulin secretion by choline and choline compounds. Furthermore, α_2 -adrenoceptor mediated hyperglycemic response to choline and choline compounds is apparently strong enough to prevent the decline in blood glucose in response to the elevation in serum insulin levels.

Gotoh et al. [8] reported that i.c.v. injection of neostigmine, a cholinesterase inhibitor, increased plasma insulin concentrations in bilaterally adrenalectomized rats. In the present study, we show that i.c.v. choline increases serum insulin concentrations that are blocked by central pretreatment with a neuronal type nicotinic receptor antagonist, mecamylamine, and with the muscarinic receptor antagonist, atropine. The increases in serum insulin concentrations evoked by central choline are also prevented by i.c.v. pretreatment with the neuronal choline uptake blocker HC-3. Taken together, it is suggested that central choline increases serum insulin by increasing central nicotinic and muscarinic cholinergic neurotransmission (as evidenced by blockade by central mecamylamine and atropine) by acting presynaptically as a precursor (as evidenced by blockade by neuronal choline uptake inhibitor) which activates vagal nerve (as evidenced by a significant bradycardia [3]) and stimulates secretion of insulin from β -cells.

In summary, the results of the present study show that intraperitoneal administration of CDP-choline or its cholinergic metabolites phosphocholine and choline elevates circulating insulin concentrations. The increases in serum insulin are associated with the increases in serum-free choline concentrations and are mediated both by peripheral muscarinic and nicotinic acetylcholine receptors. Since insulin has several important actions in the periphery as well as in the central nervous system [20], elevations in circulating insulin can mediate some of the actions of CDP-choline.

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