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Hyperglycemia induced by intracerebroventricular choline: involvement of the sympatho-adrenal system

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Abstract

Intracerebroventricular (i.c.v.) injection of choline (75–300 μg) produced a dose-dependent increase in blood glucose levels. Pre-treatment with the nicotinic acetylcholine receptor antagonist, mecamylamine (50 μg, i.c.v.) blocked the hyperglycemia induced by choline (150 μg, i.c.v.), but the response was not affected by pre-treatment with the muscarinic acetylcholine receptor antagonist, atropine (10 μg, i.c.v.). Pre-treatment with the neuronal choline uptake inhibitor, hemicholinium-3 (20 μg, i.c.v.), attenuated the hyperglycemia induced by choline. The hyperglycemic response to choline was associated increased plasma levels of adrenaline and noradrenaline. The hyperglycemia elicited by choline was greatly attenuated by bilateral adrenalectomy, and entirely blocked by either surgical transection of the splanchnic nerves or by pre-treatment with the α-adrenoceptor antagonist, phentolamine. These data show that choline, a precursor of acetylcholine, increases blood glucose and this effect is mediated by central nicotinic acetylcholine receptor activation. An increase in sympatho-adrenal activity appears to be involved in the hyperglycemic effect of choline.

Keywords: Choline; Hyperglycemia; Glucoregulation; Sympatho-adrenal activity; Cholinergic activity, rat

1. Introduction

The rates at which neurons synthesize and release the neurotransmitter acetylcholine can be affected by the levels of its precursors choline and acetyl-coenzyme A, the latter being formed from glucose (for review, see Tucek, 1993). Treatments that raise circulating and tissue choline levels cause a parallel increase in acetylcholine synthesis (Cohen and Wurtman, 1975) and release (Maire and Wurtman, 1985; Ulus et al., 1989; Koshimura et al., 1990; Johnson et al., 1992; Farber et al., 1993; Marshall and Wurtman, 1993; Buyukuysal et al., 1995) and augment cholinergic transmission (Ulus and Wurtman, 1976; Ulus et al., 1977, 1978; Wecker and Schmidt, 1979) causing functional changes in neurons and endocrine cells postsynaptic to those with elevated acetylcholine levels (Savci et al., 1996a,b; Gurun et al., 1997a). In addition to increasing acetylcholine synthesis and release, high concentrations of choline elicit biological effects by acting as an agonist for acetylcholine receptors directly (Ulus et al., 1988). Choline and choline-containing compounds have some degree of therapeutic benefit in memory loss (Spiers et al., 1996; Alvarez et al., 1997), ischemic and traumatic central nervous system injury, aging and Alzheimer's disease (Alvarez et al., 1999; Lozano, 1991; Levin, 1991; Caamano et al., 1994; Cacabelos et al., 1996; Clark et al., 1999).

Several studies have provided evidence that the acute administration of glucose stimulates acetylcholine synthesis (Dolezal and Tucek, 1982), attenuates the depletion in brain acetylcholine induced by the muscarinic antagonists, atropine, scopolamine and quinuclidinyl benzilate (Stone et al., 1988, 1991; Ricny et al., 1992). In microdialysis studies, glucose enhanced the scopolamine-induced acetylcholine over flow and facilitated acetylcholine synthesis in rat hippocampus after morphine administration (Ragozzino and Gold, 1995; Ragozzino et al., 1994). Systhemic administration of glucose increases hippocampal acetylcholine efflux and enhances performance on a spontaneous alternation task (Ragozzino et al., 1996, 1998). Intraperitoneal co-administration of glucose plus choline improves memory for a passive avoidance task and increases hippocampal acetylcholine release (Kopf et al., 2001). Glucose admin-

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istration has positive effects on learning and memory in rodents (Gold et al., 1986; Messier and Destrade, 1988; Kopf and Baratti, 1994) and humans with Alzheimer's disease (Manning et al., 1993), with Down's syndrome and in the elderly (Manning et al., 1998).

Moreover, numerous studies point a major role of central cholinergic neurons in the central regulation of blood glucose concentration. Central administration of the cholinergic agonists, carbachol (Brown et al., 1979; Brito et al., 1993), muscarine, acetycholine, methacholine and bethanecol (Iguchi et al., 1986) or an inhibitor of acetylcholinesterase neostigmine (Brito et al., 1993; Hanmura et al., 1992; Iguchi et al., 1986, 1988, 1990; Takahashi et al., 1998), cause a marked increases in blood glucose levels. The mechanisms that mediate the central cholinergic glucoregulation involve the autonomic nervous pathways that secrete glucoregulatory hormones such as adrenaline, glucagon and insulin, and directly innervate the liver (Hanmura et al., 1992; Iguchi et al., 1986, 1988).

Considering the scientific and clinical interests reflected in the great number of studies dealing with the effects of glucose and choline on cholinergic neurotransmission and the role of central cholinergic neurons in the glucoregulation, it is surprising that the effect of choline on blood glucose concentration has not yet been investigated. The goal of the present study was to investigate possible actions of choline administration on blood glucose. The present results show that i.c.v. administration of choline increases blood glucose concentrations in a dose-dependent manner. This effect is mediated by nicotinic receptors and is transmitted through a pathway that involves the activation of sympatho-adrenal outflow and secretions of catecholamines.

2. Materials and methods

2.1. Animals

Male Wistar rats (Experimental Animals Breeding and Research Center, Uludag University Medical Faculty, Bursa, Turkey) weighing 300–350 g were used in all experiments. Four rats were housed in hanging cages, given ad libitum access to food and water, and were exposed daily to regular 12 h light–dark cycle. Animals were allowed at least a week of adaptation to their new environment before use in experiments. In the experiments, all efforts were made to minimize the suffering and the numbers of animals were used. The experimental and surgical procedures were approved by the Animal Care and Use Committee of Uludag University.

2.2. Surgical procedures

Animals were anaesthetized with ether; a burr hole was drilled through the skull 1.5 mm lateral to the midline and 1.0 mm posterior to the bregma and a 10-mm length of 20 gauge stainless steel hypodermic tubing (injection guide cannula) was directed through the hole toward the right lateral cerebral ventricle. The tip of the guide cannula was lowered 5.0 mm below the skull surface and was fixed to the skull with acrylic cement. In three sets of experiments, a catheter (PE-50 tubing, 25–30 cm) filled with heparinized saline (400 U/ml) was inserted into the left common artery for repeated blood sampling. Lumbar bilateral adrenalectomy or a sham operation was performed on 17 and 12 rats, respectively. In one set of experiments, lumbar bilateral splanchnic nerve transection ($n=12$) or a sham operation ($n=11$) were performed under sodium pentobarbital (50 mg/kg, intraperitoneal) anesthesia, 3 weeks prior to the cannulation.

Following the completion of these surgical and cannulation procedures, the rats were placed in individual small plastic cages and allowed to recover from anesthesia. Observations commenced about 3 h after the rats had regained consciousness. During this observation period, rats were left undisturbed and did not show any sign of pain. In preliminary studies, we assessed the effects of ether anesthesia and/or surgical procedures on the blood glucose levels. Although there was slight but significant hyperglycemia during ether anesthesia and surgical manipulations, blood glucose levels returned to basal levels within 30 min after the anesthesia and surgery ended.

2.3. Experiments

In first series of experiment, 33 rats were used to asses the dose- and time-dependent effects of choline on serum glucose levels. Rats were injected 20 μl (i.c.v.) of saline or choline (75, 150 or 300 μg). A blood sample (0.1 ml) was withdrawn from the arterial catheter 5, 15, 30, 45, 60 and 90 min after i.c.v. choline injection for serum glucose measurements.

In the second series of experiments, rats were pretreated i.c.v. with saline (10 μl), atropine (10 μg) or mecamylamine (50 μg) 15 min before i.c.v. injection of saline (10 μl), choline (150 μg) or carbachol (10 μg). A blood sample was withdrawn from the arterial catheter immediately before each i.c.v. injection and 15, 30, 45 and 60 min after the second i.c.v. injection for serum glucose measurements.

In the third series of experiments, rats were pretreated i.c.v. with either saline (10 μl) or the neuronal choline uptake inhibitor hemicholinium-3 (20 μg) 15 min before the i.c.v. injection of saline (10 μl) or choline (150 μg). Animals were killed by rapid decapitation 15 min after the second i.c.v. injection and trunk blood was collected for serum glucose measurements.

In the fourth series of experiments, rats received i.c.v. saline (10 μl) or choline (150 μg) and a blood sample was withdrawn from the arterial catheter 15 min later for serum glucose and plasma adrenaline and noradrenaline measurements.

In the fifth series of experiments, rats were pretreated intraperitoneally with either saline (1 ml/kg), the α-adreno-

ceptor antagonist phentolamine (10 mg/kg) or the β-adrenoceptor antagonist propranolol (5 mg/kg) 15 min before the i.c.v. injection of saline (10 μl) or choline (150 μg). Animals were killed by rapid decapitation 15 min after the second i.c.v. injection and trunk blood was collected for serum glucose measurements.

In the sixth series of experiments, the hyperglycemic response to i.c.v. choline was tested in bilaterally adrenalectomized or splanchnic nerve transected rats. Bilateral adrenalectomy or sham operation was performed on the same day of the experiments, about 3 h before i.c.v. injection of saline or choline (150 μg). Bilateral splanchnic nerve transection or sham operation was performed 3 weeks prior to the experiments. On the day of the experiment, rats received saline or choline (150 μg), they were killed by rapid decapitation 15 min after the second i.c.v. injection and trunk blood was collected for serum glucose measurements.

2.4. Determinations of glucose, adrenaline and noradrenaline

After sampling, the blood was immediately stored on ice, serum or plasma separated by centrifugation and stored at −80 °C until assayed for its content of adrenaline and noradrenaline. Plasma adrenaline and noradrenaline were determined by radioenzymatic method using a commercially available kit (Amersham Pharmacia, UK), as described previously (Ulus et al., 1995).

Serum glucose levels were determined in 5 μl of serum with the glucose oxidase method using a commercially available assay kit (Biotrol, France), per manufacturer instructions.

2.5. i.c.v. Injection of drugs

For i.c.v. injection, the injection cannula (25 gauge, 11.5 mm stainless steel tubing) was inserted through the guide cannula. The injection cannula was connected to a Hamilton microsyringe (50 μl) by polyethylene tubing (PE 50), which was filled with saline (0.150 M NaCl) or saline containing the desired dose of the drug of interest in 10 or 20 μl. Drugs were then infused slowly within 6–8 s. In two sets of experiments, rats received two i.c.v. injections at a 15-min interval. In preliminary studies, we had assessed the effects of i.c.v. injection of the isotonic and the hypertonic NaCl solutions on the blood glucose levels. Those studies had shown slight but significant hyperglycemia after i.c.v injection of 20 μl of the hypertonic (0.300 M) NaCl solutions, whereas blood glucose levels had remained unchanged after i.c.v. injection of 20 μl of the isotonic (0.150 M) NaCl solution. A rapid i.c.v. injection of 20 ml of the isotonic saline (0.150 M NaCl) also produced slight and short lasting hyperglycemia. Thus, in order to avoid non-specific arousal and consequently hyperglycemia via increasing tonicity of the cerebrospinal fluid, in the study described here, the isotonocity of the solution injected i.c.v. was carefully maintained and drugs were injected i.c.v. by slow infusion within 6–8 s. When choline was injected at increasing doses, isotonicity was maintained by appropriately decreasing the concentration of NaCl in the vehicle solution.

2.6. Drugs

Choline chloride, atropine sulfate, mecamylamine hydrochloride, hexamethonium hydrochloride, hemicholinium-3 bromide, carbamylcholine hydrochloride (Sigma, St. Louis, MO, USA), phentolamine hydrochloride (Ciba-Geigy, Istanbul, Turkey) and propranolol hydrochloride were dissolved in physiological saline (0.9% NaCl), NaCl in the vehicle solution.

2.7. Statistical evaluations

Statistical evalution consisted of one- or two-way analysis of variance (ANOVA) with post hoc Tukey's test, paired and unpaired *t*-test. Data are presented as mean ± standard error of mean (S.E.M.).

3. Results

3.1. Effect of i.c.v. choline on serum glucose level

Baseline serum glucose concentration before i.c.v. choline or saline treatments was 138 ± 4 mg/100 ml ($n = 28$).

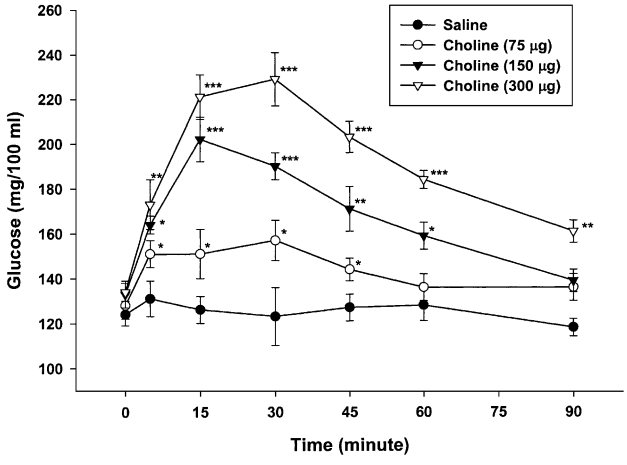

Fig. 1. Dose and time related elevation in blood glucose following i.c.v. injection of choline. Conscious freely moving rats received i.c.v. saline (20 μl) or choline (75–300 μg). Blood samples (0.2 ml) were collected from a carotic artery catheter inserted into the left carotid artery, immediately before (0) and 5, 15, 30, 45, 60 and 90 min after treatment. Serum glucose was determined by the glucose oxidase method. Each point represents the mean ± S.E.M. of 6–10 measurements. Data were analyzed with ANOVA with repeated measures and followed by Tukey's test. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with the same time point from saline controls.

Choline (75, 150 or 300 μg) increased serum glucose concentration by i.c.v. injection. The hyperglycemic response began within 5 min after choline injection and reached a maximum within 15–30 min. Glucose returned to control levels by 45–90 min depending on the choline dose (Fig. 1). The effect of choline on serum glucose increase was dose dependent, with increases of 23 ± 6, 59 ± 8 and 93 ± 13 mg/100 ml glucose following choline doses of 75, 150 or 300 μg, respectively.

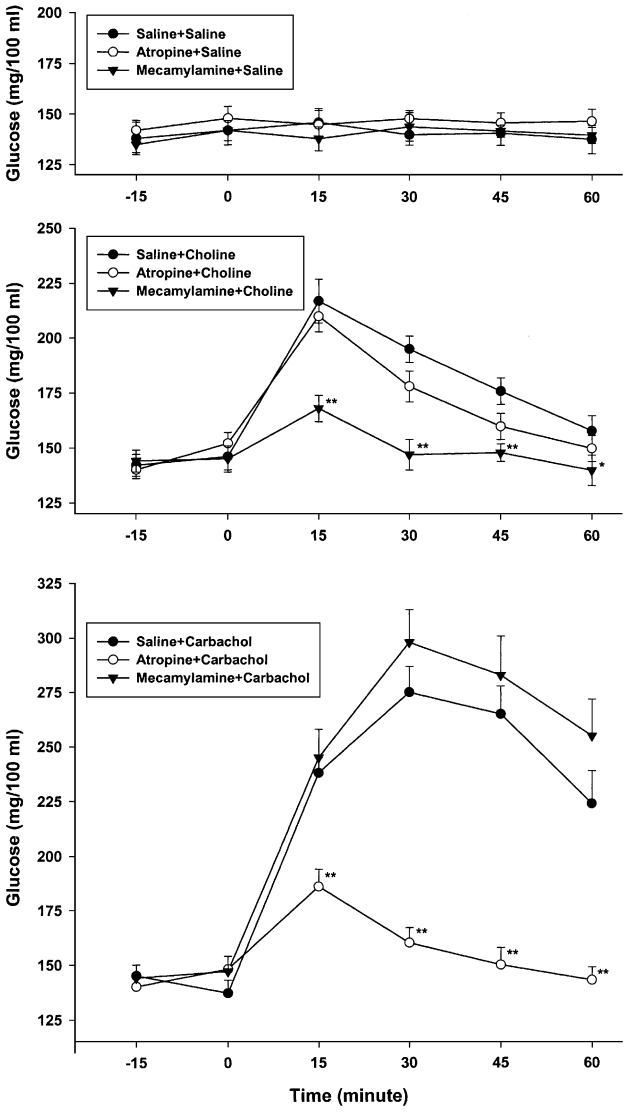

Fig. 2. Effects of atropine and mecamylamine on the hyperglycemia induced by i.c.v. choline or carbachol. Rats were treated i.c.v. with saline (10 μl), atropine (10 μg) or mecamylamine (50 μg) 15 min before i.c.v. administration of saline (10 μl), choline (150 μg) or carbachol (10 μg). Blood samples were withdrawn through the arterial catheter immediately before the first (−15) and second i.c.v. injections (0) and 15, 30, 45 and 60 min after the second i.c.v. injection for serum glucose measurement. Data are expressed as the mean ± S.E.M. of the six to nine animals. Data were analyzed by two-way ANOVA with repeated measures and followed by Tukey's test. *$P<0.05$; **$P<0.01$ compared with the samples collected at the same time point from choline or carbachol-treated rats.

Table 1
Serum glucose response to i.c.v. choline in hemicholinium-3 pretreated rats

	Glucose (mg/100 ml)		
	Before pretreatment	After pretreatment	Changes after second i.c.v. treatment
Saline + Saline	142 ± 2	142 ± 1	3 ± 1
Saline + Choline	145 ± 6	143 ± 3	64 ± 12[a]
Hemicolinium-3 + Saline	144 ± 6	176 ± 6[b]	8 ± 5
Hemicolinium-3 + Choline	139 ± 13	174 ± 5[b]	21 ± 7

Rats were pretreated with saline (10 μl) or hemicholinium-3 (20 μg) i.c.v. Fifteen minutes later, they were given saline (10 μl) or choline (150 μg) i.c.v. Arterial blood samples were obtained immediately before the first (before pre-treatment) and second i.c.v. injections (after pretreatment), and 15 min after the second i.c.v. injection for measurement of serum glucose. Data are presented as mean ± S.E.M. of six animals and were analyzed by two-way ANOVA followed by Tukey's test.

[a] $P<0.05$ compared with the changes in the "saline + saline" and the "hemicholinium-3 + choline".

[b] $P<0.05$ compared with the "before pretreatment" values.

Intra-arterial infusion of centrally effective dose (150 μg) of choline failed to affect serum glucose levels (142 ± 11 mg/100 ml; $n=6$). However, serum glucose did increase significantly ($P<0.01$) by 59 ± 5 mg/100 ml ($n=8$) from the baseline level of 147 ± 9 mg/100 ml, 15 min after intraperitoneal administration of a much higher dose of choline chloride, 120 mg/kg.

3.2. Nicotinic receptor antagonist mecamylamine blocks the hyperglycemia induced by i.c.v. choline

Rats were pre-treated i.c.v. with saline (10 μl), mecamylamine (50 μg) or atropine (10 μg) 15 min before i.c.v. administration of choline (150 μg) to determine if nicotinic and/or muscarinic receptors mediate the hyperglycemic response to choline. The nicotinic receptor antagonist mecamylamine (50 μg, i.c.v) completely blocked the increase in serum glucose produced by choline, whereas the muscarinic receptor antagonist atropine was ineffective (Fig. 2). In contrast, the pre-treatment with the same dose of mecamylamine (50 μg) failed to alter the hyperglycemic response to i.c.v. carbachol (10 μg), whereas the response was inhibited significantly by the same dose (10 μg) of atropine (Fig. 2). Neither atropine nor mecamylamine produced any effect on blood glucose levels when administered alone (Fig. 2).

3.3. Hemicholinium-3 attenuates choline-induced hyperglycemia

Rats were pre-treated with i.c.v. saline (10 μl) or hemicholinium-3 (20 μg) 15 min prior to i.c.v. choline (150 μg). Hemicholinium-3, a high affinity neuronal choline uptake inhibitor, significantly ($P<0.05$) increased blood glucose 32 ± 7 mg/100 ml above baseline (144 ± 6 mg/100 ml; $n=7$). Choline (150 μg) or saline (10 μl) injection 15 min after hemicholinium-3 produced an additional 21 ± 7 mg/100 ml ($n=7$) or 8 ± 5 mg/100 ml ($n=7$) increase, respectively

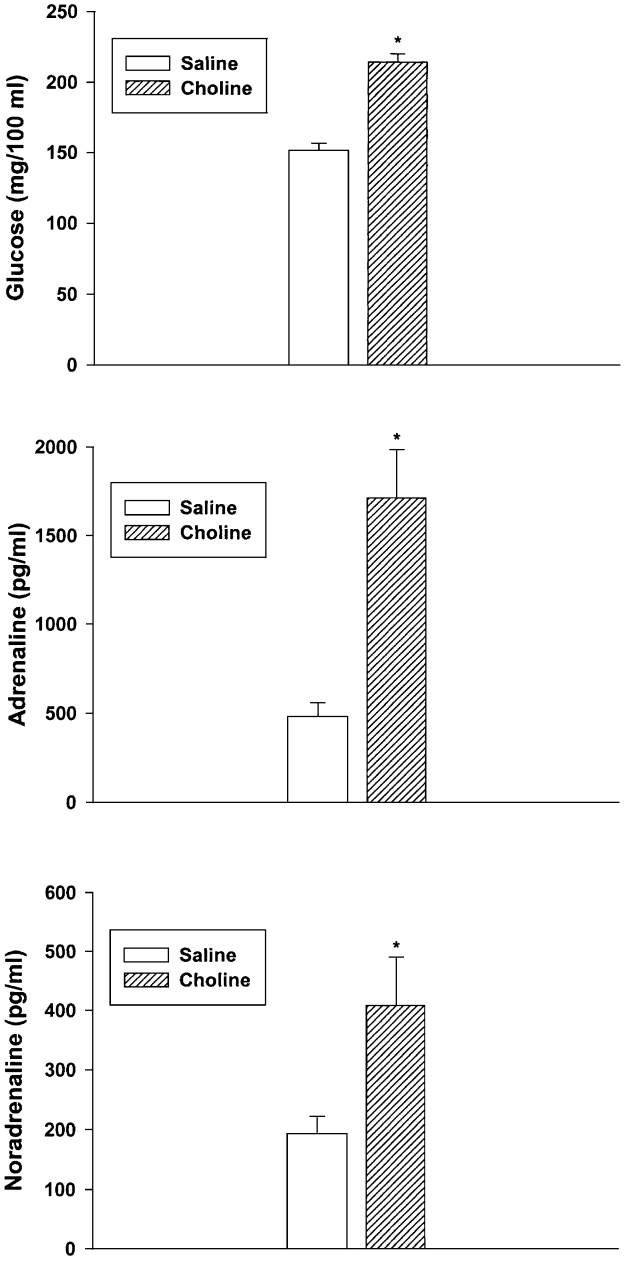

Fig. 3. Choline-induced hyperlycemia is associated with elevated plasma adrenaline and noradrenaline concentrations. Fifteen minutes after i.c.v. injection of saline (10 μl) or choline (150 μg) two consecutive blood samples were withdrawn through an arterial catheter for measurements of serum glucose and plasma adrenaline and noradrenaline concentrations. Glucose was measured by the glucose oxidase method and noradrenaline and adrenaline were measured by radio-enzimatic assay. Data represent the mean ± S.E.M. of six animals and were analyzed with Student's *t*-test. $*P < 0.01$ compared with the value from saline controls.

(Table 1). In hemicholinium-3 pre-treated animals, the increase in serum glucose in response to choline was 21 ± 7 mg/100 ml, significantly lower than the observed increase, 64 ± 12 mg/100 ml ($n = 7$), in the saline pre-treated group (Table 1).

3.4. Choline-induced hyperglycemia associates with an increase in plasma adrenaline and noradrenaline levels

Fig. 3 illustrates the concentrations of plasma glucose, adrenalin and noradrenaline 15 min after i.c.v. injection of choline (150 μg). Plasma adrenaline and noradrenaline increased significantly after i.c.v. administration of choline (Fig. 3).

3.5. Effects of pharmacological and surgical interventions of the sympatho-adrenal system functions on the hyperglycemic response to choline

To determine the involvement of the sympatho-adrenal system in the choline-induced increase in serum glucose, the peripheral sympatho-adrenal system function of rats was altered either pharmacologically or surgically before choline was administered i.c.v.

To block the effects of adrenaline and/or noradrenaline at their receptors levels, rats were pre-treated intraperitoneally with either phentolamine (10 mg/kg) or propranolol (5 mg/kg) 15 min before they received i.c.v. choline (150 μg). Pre-treatment with the specific α-adrenoceptor antagonist phentolamine totally blocked (Fig. 4) the hyperglycemic response to i.c.v. choline (150 μg) while the β-adrenoceptor antagonist propranolol was ineffective (Fig. 4). Phentolamine and propranolol, given alone, did not affect blood glucose levels.

Surgical removal of the adrenal glands 3 h before i.c.v. choline treatment, attenuated the hyperglycemic response to i.c.v. injection of 150 μg of choline significantly (Fig. 5). In adrenalectomized rats, the hyperglycemic response to choline was much smaller than the response observed in sham-operated control rats (Fig. 5).

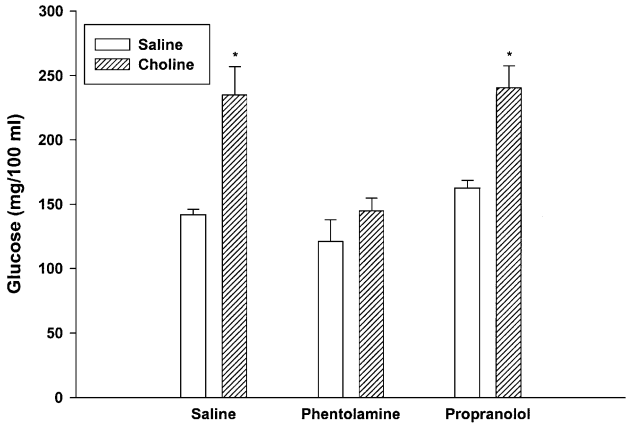

Fig. 4. Effects of phentolamine and propranolol on the hyperglycemia induced by i.c.v. choline. Saline (1ml/kg), phentolamine (10 mg/kg) or propranolol (5 mg/kg) was injected intraperitonally 15 min before i.c.v. injection of saline (10 μl) or choline (150 μg). Rats were sacrificed by decapitation 15 min later and blood was collected for measurement of serum glucose by the glucose oxidase method. Data represent the mean ± S.E.M. of six animals. Statistics were performed using ANOVA followed by Tukey's test. $*P < 0.01$ compared with the values from saline controls.

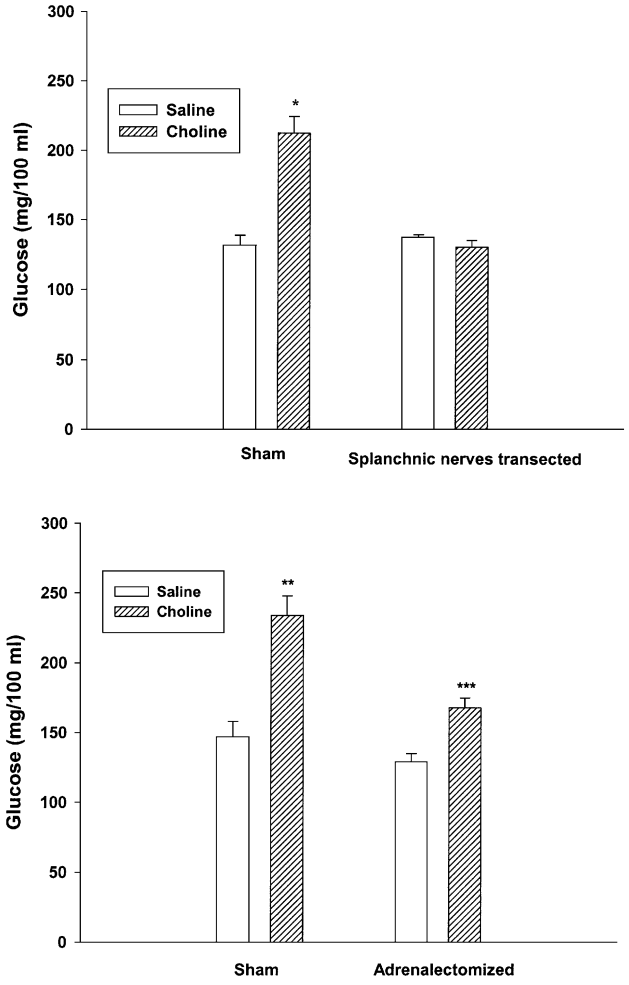

Fig. 5. Effects of adrenalectomy and splanhnic nerve transection on the hyperglycemia induced by i.c.v. choline. Bilateral splanhnic nerve transection or sham oparation was performed 3 weeks prior to the experiment. Bilateral adrenalectomy or sham operation was performed 3 h before i.c.v. administration of saline (10 μl) or choline (150 μg). On the days of the experiments, rats received i.c.v. saline (10 μl) or choline (150 μg). They were sacrificed by decapitation 15 min after i.c.v. injection of saline or choline and blood was collected for measurement of serum glucose. Data are presented as mean ± S.E.M. of 6–12 animals were analyzed ANOVA followed by Tukey's test. *$P<0.01$ compared with either saline in the sham group or choline in the splanhnic nerve transected animals. **$P<0.01$ compared with saline in the sham adrenalectomized rats. ***$P<0.05$ compared with either saline in the adrenalectomized rats or choline in the sham.

Bilateral adrenal denervation, by surgical transection of the splanchnic nerves 3 weeks before the experiments, entirely blocked the hyperglycemic response to i.c.v. choline (Fig. 5).

4. Discussion

In the present study, we showed that choline administration into the lateral cerebral ventricle of awake, unrestrained, fed rats elevates serum glucose concentration. Pre-treatment with mecamylamanine, an antagonist of nicotinic cholinergic receptors, blocked the choline-induced increase in plasma glucose. The hyperglycemic response to i.c.v. choline was not affected by pre-treatment with atropine, muscarinic cholinoceptor antagonist. The hyperglycemic response to choline was associated with significant increases in plasma insulin, glucagon, adrenaline and noradrenaline. The hyperglycemia induced by choline was blocked by bilateral transection of the splanchnic nerves or was greatly attenuated by surgical removal of adrenal glands. Antagonist of peripheral α-adrenoceptors also blocked the hyperglycemic response to choline. These findings indicate that choline produces hyperglycemia by activating central nicotinic receptors that influence adrenal catecholamine secretion.

These results complement and extend our previous studies showing that central administration of the same doses of choline to rats produces a variety of pharmacological actions; it increases blood pressure (Arslan et al., 1991), prevents hypotension (Ulus et al., 1995: Savci and Ulus, 1996, 1997, 1998; Gurun et al., 1997b), decreases body temperature (Unal et al., 1999), and elevates plasma ACTH (Savci et al., 1996b), β-endorphin (Savci et al., 1996b), prolactin (Gurun et al., 1977a), oxytocin (Savci et al., 1996a) and vasopressin (Arslan et al., 1991; Ulus et al., 1995; Savci and Ulus, 1996, 1997, 1998; Gurun et al., 1997b) concentrations, by augmenting central cholinergic neurotransmission. The observed hyperglycemia after choline is also in good agreement with previous studies from other laboratories (Brown et al., 1979; Iguchi et al., 1986, 1988; Takahashi et al., 1998; Brito et al., 1993) showing that increase central cholinergic activity produces profound hyperglycemia, and provide an another example of the or an ability of choline to produce biological effects in cholinergic nature.

In the present study, we found that the nicotinic receptor antagonist mecamylamine inhibited choline-induced hyperglycemia significantly, but atropine, a muscarinic receptor antagonist showed a very limited effect (Fig. 2). In most previous studies, atropine was found to be very effective in blocking the hyperglycemia elicited by cholinergic agents (Brown et al., 1979; Iguchi et al., 1986, 1990; Uemura et al., 1989; Takahashi et al., 1998). Conceivably, atropine may have failed to inhibit choline-induced hyperglycemia in the present study if the dose was insufficient. This is unlikely, however, because the same atropine dose was previously shown to block choline-induced hypothermia (Unal et al., 1999) and oxytocin secretion (Savci et al., 1996a). Moreover, we found that the same dose of atropine blocked carbachol-induced hyperglycemia completely. It seems, therefore, that different central acetylcholine receptors are involved in the hyperglycemic response to choline and carbachol. This view is further supported by the fact that mecamylamine failed to alter the hyperglycemic response to carbachol but effectively blocked the hyperglycemia induced by choline. It is clear from these results that choline-induced hyperglycemia is mediated primarly by nicotinic receptors. The involvement

of central nicotinic receptors in the hyperglycemic response to central cholinergic stimulation has also been reported previously (Shimazu and Ishikawa, 1981; Ishikawa et al., 1982). Muscarinic receptors play a minimal role, if any, in choline-induced hyperglycemia.

Ability of choline to increase central nicotinic cholinergic activity as reflected by the hyperglycemia might result from its precursor effect on central cholinergic neurons. This conclusion is supported by the finding that pretreating rats with high affinity choline uptake hemicholinium-3 produced its own hyperglycemic effect in our experiments, however, which makes it difficult to attribute choline's hyperglycemic effect to increased acetylcholine synthesis and release with full confidence. The mechanism responsible for the hyperglycemia produced by hemicholinium-3 is not known, but might be explained by the its acetylcholine releasing action (Poulain et al., 1987; Umeda and Sumi, 1990). Choline also activates nicotinic receptors directly (Ulus et al., 1988) in concentrations ($K_i = 375-1167$ μM), an order of magnitude greater than those (10–60 μM) known to increase acetycholine synthesis and release from cholinergic neurons (Maire and Wurtman, 1985; Ulus et al., 1989). Whether the concentration of choline at central acetylcholine receptor sites is increased high enough by i.c.v. injection of choline to make a significant contribution to the observed hyperglycemia is not known at the present time. If so, then choline may cause hyperglycemia through both pre- and post-synaptic mechanisms.

After i.c.v. injection of choline, there was not only an increase in blood glucose, but also an increase in plasma adrenalin and noradrenalin (Fig. 3). The increase in plasma plasma catecholamines were in good accordance with our previous report (Arslan et al., 1991) and indicate that choline activates the sympatho-adrenal system. The increase in plasma noradrenalin was much less than the increase in adrenaline (Fig. 3), consistent with the previous observation that central cholinergic stimulation primarily activates the adrenomedullary pathway of the sympatho-adrenal system (Ulus and Wurtman, 1979; Arslan et al., 1991).

We found that the hyperglycemia produced by choline is prevented completely by either blockade of peripheral α-adrenoceptors (Fig. 4) or bilateral transection of the splanchnic nerves (Fig. 5) and was attenuated significantly by bilateral adrenalectomy (Fig. 5). These data clearly show that activation of the sympatho-adrenal system mediates in the hyperglycemic response to choline. From these data, it is reasonable to assume that choline causes hyperglycemia by activating nicotinic receptors in the central nervous system (as blocked by mecamylamine), which activates peripheral sympatho-adrenal system through the splanchnic nerves (blocked by transection) and stimulates secretion of adrenaline and noradrenaline (which are increased in the circulation). Adrenaline and/or noradrenaline presumable stimulate α-adrenoceptors in the liver, and thus increase hepatic production of glucose (Scheurink et al., 1988), and/or in the pancreas, where they stimulate secretion of glucagon which ultimately results in a net increase in hepatic glucose production (Yamaguchi, 1992). The fact that bilateral adrenalectomy attenuated the hyperglycemic effect of central choline strongly suggest that adrenal secretion of catecholamines is essential for the elevation of blood glucose level. However, we can not conclude that the hyperglycemia is entirely dependent on adrenal gland, since a small but significant hyperglycemia was still present in adrenalectomized rats. Considering the facts that the hyperglycemia was absent in α-adrenoceptor blocked or splanchnic nerves transected rats, the remaining hyperglycemia in adrenalectomized rats is attributable to the direct neural activation of α-adrenoceptors, in the liver and/or pancreas, through the splanchnic nerves. The observed hyperglycemia in adrenalectomized rats could have occurred also through adrenaline, released from the sympathetic nerve terminal, as it is taken up and stored in sympathetic nerve terminals as a co-transmitter with noradrenaline (Coppes et al., 1994, 1995). This view supported by the observation is that a small but significant plasma adrenaline response was still present in adrenalectomized rats (Gürün et al., unpublished observation).

The central site(s) for choline-induced hyperglycemia remains to be identified. Although several areas within the central nervous system influence sympatho-adrenal activity, ventromedial hypothalamic cholinergic neurons (apparently via nicotinic receptors) are known to have an has an important role in the central regulation of carbohydrate metabolism, largely mediated through sympatho-adrenomedullary activation (Shimazu and Ishikawa, 1981; Ishikawa et al., 1982). It has been shown that choline is widely distributed in the brain following i.c.v. administration and that it increases acetylcholine synthesis in several brain regions, including the hypothalamus (Buccafusco, 1982). Thus, it is likely that choline elevates blood glucose levels by increasing nicotinic cholinergic transmission in the ventromedial hypothalamus.

While the ability of choline to increase cholinergic function is well known, the results from the present study provide the first evidence that choline elevates blood glucose levels through a central mechanism.

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References

Alvarez, X.A., Laredo, M., Corzo, D., Fernadez-Novoa, L., Mouzo, R., Perea, J.E., Daniele, D., Cacabelos, R., 1997. Citicoline improves memory performance in elderly subjects. Methods Find. Exp. Clin. Pharmacol. 19, 201–210.

Alvarez, X.A., Mouzo, R., Pichel, V., Perez, P., Laredo, M., Fernadez-Novoa, L., Corzo, L., Zas, R., Alcaraz, M., Secades, J.J., Lozano, R., Cacabelos, R., 1999. Double-blind placebo-controlled study with citicoline in APOE genotyped Alzheimer's disease patients. Effects on cognitive performance, brain bioelectrical activity and cerebral perfusion. Methods Find. Exp. Clin. Pharmacol. 21, 633–644.

Arslan, B.Y., Ulus, I.H., Savci, V., Kiran, B.K., 1991. Effects of intracerebroventricular injected choline on cardiovascular functions and sympathoadrenal activity. J. Cardiovasc. Pharmacol. 17, 814–821.

Brito, N.A., Brito, M.N., Kettelhut, C.I., Migliorini, R.H., 1993. Intra-vetromedial hypothalamic injection of cholinergic agents induces rapid hyperglycemia, hyperlactatemia and gluconeogenesis activation in fed, conscious rats. Brain Res. 626, 339–342.

Brown, M., Rivier, J., Vale, W., 1979. Somatostatin: central nervous system actions on glucoregulation. Endocrinology 104, 1709–1715.

Buccafusco, J.J., 1982. Kinetics of [^{3}H]choline and [^{3}H]acetylcholine metabolism in several regions of rat brain following intracerebroventricular injection of [^{3}H]choline. Effects of haloperidol. Biochem. Pharmacol. 31, 1599–1605.

Buyukuysal, R.L., Ulus, I.H., Aydin, S., Kiran, B.K., 1995. 3,4-Diaminopyridine and choline increases in vivo acetylcholine release in rat striatum. Eur. J. Pharmacol. 281, 179–185.

Caamano, J., Gomez, M.J., Franco, A., Cacabelos, R., 1994. Effects of CDP-choline on cognition and cerebral hemodynamics in patients with Alzheimer's disease. Methods Find. Exp. Clin. Pharmacol. 16, 211–218.

Cacabelos, R., Caamano, J., Gomez, M.J., Fernandez-Novoa, L., Franco-Maside, A., Alvarez, X.A., 1996. Therapeutics effects of CDP-choline in Alzheimer's disease. Cognition, brain mapping, cerebrovascular hemodynamics, and immune factors. Ann. N.Y. Acad. Sci. 17, 399–403.

Clark, W.M., Williams, B.J., Selzer, K.A., Zweifler, R.M., Sabunjian, L.A., Gammans, R.E., 1999. A randomized efficacy trial citicoline in patients with acute ischemic stroke. Stroke 30, 2592–2597.

Cohen, E.L., Wurtman, R.J., 1975. Brain acetylcholine: control by dietary choline. Science 191, 561–562.

Coppes, R.P., Brouwer, F., Freie, I., Smit, J., Zaagsma, J., 1994. Sustained prejunctional facilitation of noradrenergic neurotransmission by adrenaline as a co-transmitter in the portal vein of freely moving rats. Br. J. Pharmacol. 113, 342–344.

Coppes, R.P., Smit, J., Benthem, L., Van der Leest, J., Zaagsma, J., 1995. Co-released adrenaline markedly facilitates noradrenaline overflow through prejunctional beta 2-adrenoceptors during swimming exercise. Eur. J. Pharmacol. 274, 33–40.

Dolezal, V., Tucek, S., 1982. Effects of choline and glucose on atropine-induced alterations of acetylcholine synthesis and content in the caudate nuclei of rats. Brain Res. 240, 285–293.

Farber, S.A., Kischka, U., Marsall, D.L., Wurtman, R.J., 1993. Potentiation by choline of basal and electrically-evoked acetylcholine release, as studied using a novel device which both stimulates and perfuses rat corpus striatum. Brain Res. 607, 177–184.

Gold, P.E., Vogt, J., Hall, J.L., 1986. Glucose effect of memory: behavioral and pharmacological characteristics. Behav. Neural Biol. 46, 145–155.

Gurun, M.S., Savci, V., Kiran, B.K., Ulus, I.H., 1997a. Centrally administered choline increases plasma prolactin levels in conscious rats. Neurosci. Lett. 232, 79–82.

Gurun, M.S., Savci, V., Ulus, I.H., 1997b. Intracerebroventricular choline reverses hypotension induced by acute chemical symapthectomy. J. Auton. Pharmacol. 17, 155–163.

Hanmura, A., Yanase, M., Saito, H., Iguchi, A., 1992. Effect of intrahypothalamic injection of neostigmine on the secretion of epinephrine and norepinephrine and on plasma glucose level. Endocrinology 130, 2997–3002.

Iguchi, A., Gotoh, M., Matsunaga, H., Yatomi, A., Honmura, A., Yanase, M., Sakamoto, N., 1986. Mechanism of central hyperglycemic effect of cholinergic agonists in fasted rats. Am. J. Physiol. 251, E431–E437.

Iguchi, A., Gotoh, M., Matsunaga, H., Yatomi, A., Honmura, A., Yanase, M., Sakamoto, N., 1988. Relative contributions of the nervous system and hormones to CNS-mediated hyperglycemia. Am. J. Physiol. 251, E431–E437.

Iguchi, A., Yatomi, A., Gotoh, M., Matsunaga, H., Uemura, K., Miura, H., Satake, T., Tamagawa, T., Sakamoto, N., 1990. Neostigmin-induced hyperglycemia is mediated by central muscarinic receptor in fed rats. Brain Res. 507, 295–300.

Ishikawa, K., Suziki, M., Shimazu, T., 1982. Effects of acetylcholine injection into the hypothalamus on the insulin and glucagon release. Neuroendocrinology 34, 310–314.

Johnson, D.A., Ulus, I.H., Wurtman, R.J., 1992. Caffeine potentiates the enhancement by choline of striatal acetylcholine release. Life Sci. 51, 1597–1601.

Kopf, S.R., Baratti, C.M., 1994. Memory-improving actions of glucose: involvement of a central cholinergic mechanism. Behav. Neural Biol. 62, 237–243.

Kopf, S.R., Buchholzer, M.L., Hilgert, M., Löffelholz, K., Klein, J., 2001. Glucose plus choline improve passive avoidance behaviour and increase hippocampal acetylcholine release in mice. Neuroscience 103, 365–371.

Koshimura, K., Miwa, S., Lee, K., Hayashi, Y., Hasegawa, H., Hamahata, K., Fujiwara, M., Kimura, M., Itokawa, Y., 1990. Effects of choline administration on in vivo release and biosynthesis of acetylcholine in the rat striatum as studied by in vivo brain microdialysis. J. Neueochem. 54, 533–539.

Levin, H.S., 1991. Treatment of postconcussional symptoms with CDP-choline. J. Neurol. Sci. 103, S39–S42 (suppl).

Lozano, R., 1991. CDP-choline in the treatment of cranio-cephalic trauma. J. Neurol. Sci. 103, S43–S47 (suppl).

Maire, J.-C.E., Wurtman, R.J., 1985. Effects of electrical stimulation and choline availability on the release and contents of acetylcholine and choline in superfused slices from rat striatum. J. Physiol. (Paris) 80, 189–195.

Manning, C.A., Ragozzino, M.E., Gold, P.E., 1993. Glucose enhancement of memory in patients with probable senile dementia of the Alzheimer type. Neurobiol. Aging 14, 523–528.

Manning, C.A., Honn, V.J., Stone, W.S., Jane, J.S., Gold, P.E., 1998. Glucose effects on cognition in adults with Down's syndrome. Neuropsychology 12, 479–484.

Marshall, D.L., Wurtman, R.J., 1993. Effect of choline on basal and stimulated acetylcholine release: an in vivo microdialysis study using a low neostigmine concentration. Brain Res. 269, 269–274.

Messier, C., Destrade, C., 1988. Improvement of memory for an operant response by post-traning glucose in mice. Behav. Brain Res. 31, 185–191.

Poulain, B., Fossier, P., Baux, G., Tauc, L., 1987. Hemicholinium-3 facilitates the release of acetylcholine by acting on presynaptic nicotinic receptors at a central synapse in Aplysia. Brain Res. 435, 63–70.

Ragozzino, M.E., Gold, P.E., 1995. Glucose injections into the medial septum reverse the effects of intraseptal morphine infusion on hippocampal acetylcholine output and memory. Neuroscience 68, 981–988.

Ragozzino, M.E., Wenk, G.L., Gold, P.E., 1994. Glucose attenuates a morphine-induced decrease in hippocampal acetylcholine output: an in vivo microdialysis study in rats. Brain Res. 655, 77–82.

Ragozzino, M.E., Unick, K.E., Gold, P.E., 1996. Hippocampal acetylcholine release during memory testing in rats: augmentation by glucose. Proc. Natl. Acad. Sci. U.S.A. 93, 4693–4698.

Ragozzino, M.E., Pal, S.N., Unick, K., Stefani, M.R., Gold, P.E., 1998. Modulation of hippocampal acetylcholine release and spontaneous alternation scores by intrahippocampal glucose injections. J. Neurosci. 18, 1595–1601.

Ricny, J., Tucek, S., Novakova, J., 1992. Acetylcarnitine, carnitine and glucose diminish the effect of muscarinic antagonist quinuclidinyl benzilate on straiatal acetylcholine content. Brain Res. 576, 215–219.

Savci, V., Ulus, I.H., 1996. Central choline reverses hypotension caused by α-adrenaceptor or ganglion blockade in rats: the role of vasopressin. Eur. J. Pharmacol. 311, 153–161.

Savci, V., Ulus, I.H., 1997. Cardiovascular effects of central choline during endotoxin shock in the rat. J. Cardiovasc. Pharmacol. 30, 667–675.

Savci, V., Ulus, I.H., 1998. Choline administration reverses hypotension in spinal cord transected rats: the involvement of vasopressin. Neurochem. Res. 23, 733–741.

Savci, V., Gurun, S., Ulus, I.H., Kiran, B.K., 1996a. Intracerebroventricular injection of choline increases plasma oxytocin levels in concious rats. Brain Res. 709, 97–102.

Savci, V., Gurun, M.S., Ulus, I.H., Kiran, B.K., 1996b. Effect of intracerebroventricularly injected choline on plasma ACTH and β-endorphin levels in conscious rats. Eur. J. Pharmacol. 309, 275–280.

Scheurink, A.J.W., Steffens, A.B., Benthem, L., 1988. Central and peripheral adrenoceptors affect glucose, free fatty acids, and insulin in exercising rats. Am. J. Physiol. 255, R547–R556.

Shimazu, T., Ishikawa, K., 1981. Modulation by the hypothalamus of glucagon and insulin secretion in rabbits: studies with electrical and chemical stimulation. Endocrinology 108, 605–611.

Spiers, P.A., Myers, D., Hochanadel, G.S., Lieberman, H.R., Wurtman, R.J., 1996. Citicoline improves verbal memory in aging. Arch. Neurol. 53, 441–448.

Stone, W.S., Croul, C.E., Gold, P.E., 1988. Attenuation of scopolamine induced amnesia in mice. Psychopharmacology 96, 417–420.

Stone, W.S., Walser, B., Gold, S.D., Gold, P.E., 1991. Scopolamine-and morphin-induced impairments of spontaneous alternation performance in mice: reversal with glucose and with cholinergic and adrenergic agonists. Behav. Neurosci. 105, 264–271.

Takahashi, A., Kishi, E., Ishimaru, H., Ikarashi, Y., Maruyama, Y., 1998. Stimulation of rat hypothalamus by microdilaysis with K^+: increase of ACh release elevates plasma glucose. Am. J. Physiol. 275, R1647–R1653.

Tucek, S., 1993. Short-term control of the synthesis of acetylcholine. Prog. Biophys. Mol. Biol. 60, 59–69.

Uemura, K., Iguchi, A., Yatomi, A., Miura, H., Honmura, A., Yanase, M., Sakamoto, N., 1989. Involvement of the hippocampus in central nervous system-mediated glucoregulation in rats. Endocrinology 124, 2449–2455.

Umeda, Y., Sumi, T., 1990. Release of endogenous acetylcholine from rat brain slices with or without cholinesterase inhibition and its potentiation by hemicholinum-3. Neurosci. Lett. 118, 276–278.

Unal, C.B., Demiral, Y., Ulus, I.H., 1999. The effects of choline on body temperature in conscious rats. Eur. J. Pharmacol. 363, 121–126.

Ulus, I.H., Wurtman, R.J., 1976. Choline admnistration: activation of tyrosine hydroxylase in dopaminergic neurons of rat brain. Science 194, 1060–1061.

Ulus, I.H., Wurtman, R.J., 1979. Selective response of rat peripheral sympathetic nervous system to various stimuli. J. Physiol. (London) 293, 513–523.

Ulus, I.H., Hirsch, M.J., Wurtman, R.J., 1977. Trans-synaptic induction of adrenomedullary tyrosine hydroxylase activity by choline: evidence that choline administration can increase cholinergic transmission. Proc. Natl. Acad. Sci. U.S.A. 74, 798–800.

Ulus, I.H., Scally, M.C., Wurtman, R.J., 1978. Enhancement by choline of the induction of adrenal tyrosine hydroxylase by phenoxybenzamine, 6-hydroxydopamine, insulin and exposure to cold. J. Pharmacol. Exp. Ther. 204, 676–682.

Ulus, I.H., Millington, W.R., Buyukuysal, R.L., Kiran, B.K., 1988. Choline as an agonist: determination of its agonistic potency on cholinergic receptors. Biochem. Pharmacol. 37, 2747–2755.

Ulus, I.H., Wurtman, R.J., Mauron, C., Blusztajn, J.K., 1989. Choline increases acetylcholine release and protects against the stimulation-induced decrease in phosphatide levels within membranes of rat corpus striatum. Brain Res. 484, 217–227.

Ulus, I.H., Arslan, B.Y., Savci, V., Kiran, B.K., 1995. Restoration blood pressure by choline treatment in rats made hypotensive by haemorrhage. Br. J. Pharmacol. 116, 1911–1917.

Wecker, L., Schmidt, D.E., 1979. Central cholinergic function: relationship to choline administration. Life Sci. 25, 375–384.

Yamaguchi, N., 1992. Sympathoadrenal system in neuroendocrine control of glucose: mechanisms involved in the liver, pancreas, and adrenal gland under hemorrhagic and hypoglycemic stress. Can. J. Physiol. Pharmacol. 70, 167–206.