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Effect of intracerebroventricularly injected choline on plasma ACTH and β-endorphin levels in conscious rats

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Abstract

In the present study, we examined the effect of intracerebroventricularly injected choline on plasma ACTH (adrenocorticotrophin) and β-endorphin levels in conscious rats. The intracerebroventricularly injection of choline (50–150 μg) elevated plasma ACTH levels in a dose-dependent manner. Plasma β-endorphin levels were also significantly increased. Pretreatment of rats with mecamylamine (50 μg; intracerebroventricularly), the nicotinic receptor antagonist, completely inhibited the ACTH and β-endorphin response to choline (150 μg; intracerebroventricularly). An antagonist of the muscarinic receptor, atropine (10 μg; intracerebroventricularly), failed to alter these effects. Pretreatment of rats with hemicholinium-3 (20 μg; intracerebroventricularly), a drug which inhibits the uptake of choline into cholinergic neurons, abolished the choline-induced increases in both plasma ACTH and β-endorphin levels. These results indicate that choline can increase plasma concentrations of ACTH and β-endorphin through the activation of central nicotinic acetylcholine receptors.

Keywords: Choline; ACTH (adrenocorticotrophin); β-Endorphin; Atropine; Mecamylamine; Hemicholinium-3

1. Introduction

Choline, the precursor of acetylcholine, is the major determinant of the rates at which neurons synthesize and release the neurotransmitter acetylcholine. Since the macromolecules that transfer choline from plasma to cholinergic brain neurons and the enzyme that controls acetylcholine biosynthesis are both unsaturated at physiologic choline levels (Wurtman et al., 1981; Blusztajn and Wurtman, 1983), brain acetylcholine synthesis and content are closely regulated by the availability of extracellular choline. Studies have clearly shown that administration of choline can cause parallel increases in rat brain acetylcholine synthesis in vivo (Trommer et al., 1982; Wecker, 1986, Wecker et al., 1989) and can enhance acetylcholine release both in vitro (Maire and Wurtman, 1985; Ulus et al., 1989; Buyukuysal et al., 1991) and in vivo (Koshimura et al., 1990; Johnson et al., 1992; Farber et al., 1993; Marshall and Wurtman, 1993). Moreover, this treatment, by enhancing cholinergic transmission, can cause functional changes in neurons and endocrine cells postsynaptic to those with elevated acetylcholine levels (Ulus and Wurtman, 1976; Ulus et al., 1977; Arslan et al., 1991) and exerts various pharmacological effects. Some of these pharmacological effects have been reported recently (Arslan et al., 1991; Ulus et al., 1995). However, our knowledge regarding the pharmacological consequences of choline administration is still incomplete. For instance, there has been no study of the effect of choline on hormone(s) release from the anterior pituitary.

Both ACTH (adrenocorticotrophin) and β-endorphin are synthesized in corticotropic cells of the anterior pituitary. They are derived from a common precursor molecule which has been named proopiomelanocortin and are secreted concomitantly in response to several stimuli. The synthesis and release of these peptides from the anterior pituitary are regulated by multiple factors, including glucocorticoids, various neuropeptides and neurotransmitters. Putative neurotransmitter involvement in the release of these and other pituitary peptides has been extensively investigated in vivo and in vitro. There is substantial evidence from these studies suggesting that the cholinergic system has an important role in the regulation of the hypothalamic-pituitary-adrenal axis. Cholinergic innervation of the hypothalamo-pituitary system has been demonstrated and the localization of acetylcholine receptors in this system has been investigated (Burt and Taylor, 1980;

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Mason et al., 1983; Mason, 1985; Michels et al., 1986). It has also been reported that administration of directly acting cholinergic agonists, including nicotine, arecoline, or cholinesterase inhibitors, including physostigmine, soman, diisopropylfluorophosphate, produce significant increases in plasma ACTH and β-endorphin levels (Sithichoke and Marotta, 1978; Risch et al., 1986; Fletcher et al., 1989; Weidenfeld et al., 1989; Matta et al., 1990; Smallridge et al., 1991).

In the light of these reports and considering the importance of the precursor choline on cholinergic transmission and some functional or pharmacological consequences of choline administration, we suggested that choline can affect the secretion of ACTH and β-endorphin. In the present study, therefore, we investigated the effect of intracerebroventricularly injected choline on plasma ACTH and β-endorphin levels in conscious rats.

2. Materials and methods

All experiments were performed on adult male Wistar rats weighing between 300–350 g (Experimental Animals Breeding and Research Center, Uludag University, Medical Faculty, Bursa, Turkey). They were housed in the animal room, 4–6 per cage (22–23°C) under artificial illumination between 08:00 and 20:00 h, and had free access to food and tap water. Experimental protocols were approved by the Animal Care and Use Committee of Uludag University.

2.1. Surgical procedures

Surgical procedures were conducted under light ether anesthesia. Intracerebroventricularly cannulas were implanted into the left lateral cerebral ventricle for drug injections. A burr hole was drilled through the skull 1.5 mm lateral to the midline and 1.0 mm posterior to bregma, and a 10-mm length of stainless steel hypodermic tubing was directed through the hole toward the lateral cerebral ventricle. The cannula was lowered 4.2 mm below the skull and fixed to the skull with acrylic sement. An arterial catheter (PE50 tubing) filled with heparinized saline (200 U/ml) was inserted into the left common carotid artery for blood sampling.

At the end of these surgical procedures, rats were placed in individual small plastic cages and allowed to recover from anesthesia for 3–4 h.

2.2. Experimental procedures

Choline, atropine, mecamylamine and hemicholinium-3 were freshly prepared and were dissolved in saline (0.9% NaCl) solution. All injections were administered intracerebroventricularly in a final fluid volume of 10 μl. All choline dosages are expressed as the free base. When choline was injected in increasing doses, the isotonicity of the injection solution was maintained by decreasing the concentration of saline solution.

For time-response curves, animals received an injection of saline (10 μl) or choline (150 μg/10 μl) and 2-ml blood samples were collected 2, 5, 10, 20, 40 and 60 min postinjection; different sets of animals were used for each time interval. For dose-response curves, rats received an injection of saline or 50, 100 and 150 μg choline and blood samples were collected 5 min later. Atropine (10 μg/10 μl), mecamylamine (50 μg/10 μl) or hemicholinium-3 (20 μg/10 μl) was injected 15 min before saline or choline (150 μg) administration and blood samples were obtained 5 or 10 min after choline injection.

2.3. Measurement of plasma ACTH and β-endorphin

In order to determine plasma ACTH and β-endorphin levels, blood samples were collected carefully without causing the animal any stress. Tubes containing 50 mg EDTA were placed on ice immediately. After centrifugation at 4°C, 1500x *g*, for 15 min, plasma was separated and stored at −20°C for a few days. ACTH was directly measured in 0.1 ml of plasma by radioimmunoassay using a commercially available kit (DPC, CA, USA). Before being measured by radioimmunoassay, β-endorphin was extracted and concentrated from plasma using an affinity gel extraction method according to the procedure provided with the radioimmunoassay kit for plasma β-endorphin (INCSTAR corp., MN, USA; cat#46065). Briefly, 1 ml of plasma was applied to a column containing 0.5 ml of sepharose particles coupled with rabbit anti-β-endorphin. The top and the bottom of the columns were tightly capped and the columns were rotated end over end for 4 h in the cold room (4°C) to mix the sepharose particles with plasma. At the end of this period the columns were uncapped and plasma was allowed to drain through the column. The column was then washed with 3 × 1 ml of 0.85% saline. Adsorbed β-endorphin was eluted by rinsing the entire surface of the lower column with 0.5 ml (2 × 0.250 ml) of 0.025 N HCl. Two aliquots (0.5 ml) of eluate were used for the measurement of β-endorphin by radioimmunoassay. This extraction procedure provided 94 ± 5% (mean ± SEM; $n = 9$) recovery of unlabelled β-endorphin. The minimum detectable amount was 8 pg/ml for ACTH and 3 pmol/l for β-endorphin. When necessary, serial dilutions were made to ensure the amount of β-endorphin in the sample fell in the range of the standard curve. Both ACTH and β-endorphin antisera were highly specific for these peptides, with an extremely low (< 0.001–0.5%) cross-reactivity with other compounds (methionine enkephalin, leucine enkephalin, α-melanocyte-stimulating hormone, substance P, etc).

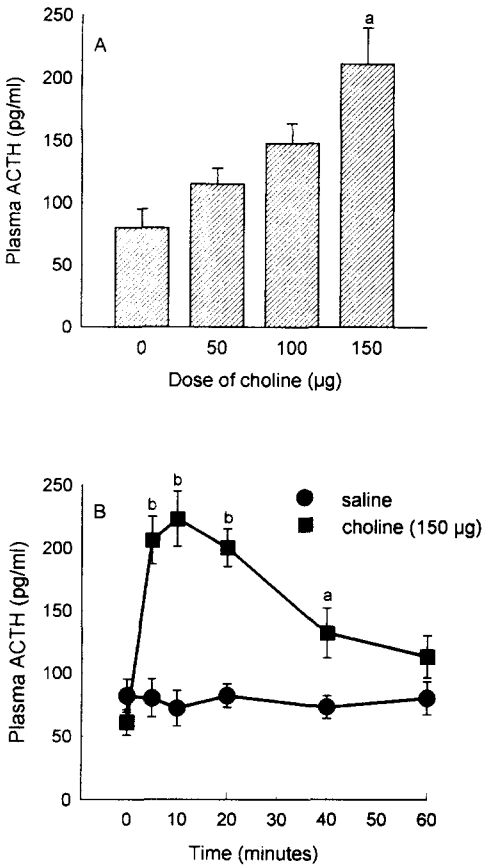

Fig. 1. (A) Effect of intracerebroventricularly injection of choline on plasma ACTH levels. Dose-response relationships. Rats were given saline (10 μl) or various doses of choline (50–150 μg/10 μl) intracerebroventricularly and blood samples were collected through the arterial catheter 5 min after these injections. (B) Time-dependent increase in plasma ACTH levels after the intracerebroventricularly injection of choline. Rats were injected with saline (10 μl) or choline (150 μg/10 μl) intracerebroventricularly and blood samples were obtained at 2, 5, 10, 20, 40 and 60 min after the injections. Blood samples were collected in ice-cold polypropylene tubes containing EDTA (50 mg) and plasma was separated immediately. Plasma ACTH was measured by RIA. Data represent means ± S.E.M. from 6–8 rats. Statistics were performed using an ANOVA with post-hoc Newman-Keuls test. (A) [a] $P < 0.01$ compared to basal levels. (B) [a] $P < 0.05$; [b] $P < 0.01$ (values significantly different from corresponding controls at the same time point).

2.4. Drugs

Choline chloride, atropine sulphate and mecamylamine HCl were obtained from Sigma (Sigma Chem. Co./St. Louis, MO, USA) and hemicholinium-3 was purchased from Aldrich (Aldrich Chem. Co., Milwaukee, WI, USA).

2.5. Data analysis

All data are presented as the means ± S.E.M. Statistical analysis of the data was performed with either Student's *t*-test or ANOVA (analysis of variance) with post-hoc Newman-Keuls test, where appropriate.

3. Results

3.1. Effect of intracerebroventricularly injected choline on plasma ACTH and β-endorphin levels; time- and dose-response relationships

In the present study, control plasma ACTH levels were 82 ± 5 pg/ml ($n = 24$). A 2.5-fold increase in plasma ACTH levels was observed after the intracerebroventricularly injection of 150 μg choline while 50 and 100 μg choline caused 1.4- and 1.8-fold increases in levels of this hormone, respectively (Fig. 1A). Fig. 1B shows the time course of the effect. Maximal increases were obtained within 10 min after 150 μg of choline administration and plasma ACTH levels returned to control values at the end of the 60-min period.

Control plasma β-endorphin levels were 65 ± 7 pmol/l

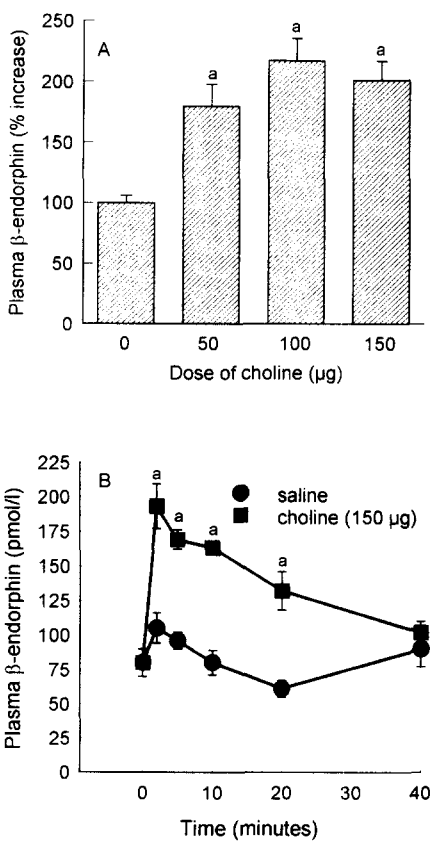

Fig. 2. (A) Changes in plasma β-endorphin levels after intracerebroventricularly choline injection. Dose-response relationships. Rats were injected with saline (10 μl) or various doses of choline (50–150 μg /10 μl) and blood samples were collected 5 min after the injections. Plasma β-endorphin levels were measured by RIA. Data represent means ± S.E.M. from 6–8 rats. [a] $P < 0.01$ significantly different from control values. (B) Time-response relationship. Choline (150 μg/10 μl) was injected intracerebroventricularly and blood samples were collected at 2, 5, 10, 20 and 40 min after injections. Plasma β-endorphin levels were determined by RIA after extraction. Data represent means ± S.E.M. from 6 rats. Statistics were performed using an ANOVA with post-hoc Newman-Keuls test. [a] $P < 0.01$ compared to corresponding controls at the same time point.

Table 1
Influence of atropine, mecamylamine or hemicholinium-3 pretreatment on plasma ACTH and β-endorphin responses to intracerebroventricularly injected choline

Group	ACTH (pg/ml)	β-endorphin (pmol/l)
Saline + saline	86 ± 17	54 ± 8
Saline + choline	191 ± 17 [b]	92 ± 9 [b]
Atropine + saline	93 ± 10	58 ± 6
Atropine + choline	172 ± 16 [b]	109 ± 12 [a]
Mecamylamine + saline	83 ± 10	63 ± 5
Mecamylamine + choline	94 ± 18	70 ± 10
Hemicholinium-3 + saline	124 ± 16	113 ± 12
Hemicholinium-3 + choline	131 ± 11	127 ± 24

Rats were pretreated with atropine (10 μg/10 μl), mecamylamine (50 μg/10 μl), hemicholinium-3 (20 μg/10 μl) or saline (10 μl) intracerebroventricularly 15 min before choline (150 μg/10 μl) injection. Blood samples were obtained 5 min after the second injection and plasma ACTH and β-endorphin levels were measured by radioimmunoassay. Data represent means ± S.E.M. from 7–8 rats. Statistical analyses of data were done by using unpaired Student's t test. [a] $P < 0.05$; [b] $P < 0.01$, significantly higher than respective control levels.

($n = 12$). The intracerebroventricularly injection of three doses of choline (50, 100 and 150 μg) produced similar and significant increases in plasma β-endorphin levels (Fig. 2A). The plasma β-endorphin response to 150 μg choline injection reached a maximum within 25 min and returned to control values at 40 min (Fig. 2B).

3.2. Effect of mecamylamine and atropine pretreatment on ACTH and β-endorphin responses to intracerebroventricularly injected choline

In order to determine the muscarinic and/or nicotinic acetylcholine receptors mediating the effect of choline on plasma ACTH and β-endorphin levels, rats were pretreated with atropine (10 μg; intracerebroventricularly), nonspecific muscarinic receptor antagonist, or mecamylamine (50 μg; intracerebroventricularly), nicotinic receptor antagonist, 15 min before the intracerebroventricular injection of 150 μg choline. Neither atropine nor mecamylamine pretreatment alone changed basal levels of the hormones. Mecamylamine totally blocked the increases in plasma ACTH and β-endorphin levels after choline injection, but atropine did not alter the response (Table 1).

3.3. Effect of hemicholinium-3 on ACTH and β-endorphin responses to intracerebroventricularly injected choline

In order to determine whether choline's effect is mediated through the presynaptic mechanisms, by increasing the synthesis and release of acetylcholine, rats were pretreated with hemicholinium-3 (20 μg; intracerebroventricularly), a specific high-affinity choline uptake inhibitor, 15 min before intracerebroventricularly choline injection (150 μg). Hemicholinium-3 pretreatment totally abolished the hormone responses to choline (Table 1). However, this pretreatment, alone, increased plasma ACTH and β-endorphin levels significantly. Previously, the facilitatory effect of hemicholinium-3 on the release of acetylcholine has been reported (Poulain et al., 1987). Our observation could be explained by this facilitatory effect of hemicholinium-3.

4. Discussion

These data show that intracerebroventricularly injected choline increases plasma ACTH and β-endorphin levels through the activation of central nicotinic receptors. Plasma ACTH levels increased gradually with increasing doses of choline while β-endorphin levels showed similar increases with all doses. The effect of choline on plasma ACTH and β-endorphin levels was time-dependent. Significant increases were observed within 5–10 min and lasted 40–60 min after choline injection. These results are consistent with earlier studies demonstrating that centrally acting cholinomimetic drugs, including the cholinesterase inhibitor physostigmine, soman, diisopropylfluorophosphate and the acetylcholine receptor agonists nicotine, arecoline, activate the hypothalamo-hypophyseal system and cause an increase in plasma ACTH and β-endorphin in humans and rats (Sithichoke and Marotta, 1978; Risch et al., 1986; Fletcher et al., 1989; Weidenfeld et al., 1989; Matta et al., 1990; Smallridge et al., 1991).

Several lines of experimental evidence suggest that both muscarinic and nicotinic acetylcholine receptors are involved in the stimulatory effects of cholinomimetics on this system (Janowsky et al., 1986; Risch et al., 1986; Weidenfeld et al., 1989; Matta et al., 1990). It has been reported that muscarinic receptors are involved in the stimulatory effect of cholinesterase inhibitors while nicotinic receptors mediate the excitatory effect of acetylcholine and nicotine. In the present study, blockade of the increase in plasma ACTH and β-endorphin in response to intracerebroventricularly injected choline with the pretreatment of rats with mecamylamine indicates the involvement of central nicotinic receptors in this effect.

Intracerebroventricularly injected choline widely distributes and converts to acetylcholine in the various brain regions, including the hypothalamus (Buccafusco, 1982; Koshimura et al., 1990). Observations from our laboratory indicate the rapid distribution of radiolabelled choline in the hypothalamus and corpus striatum within 5 min following its intracerebroventricular injection and a 2-fold increase in extracellular choline and acetylcholine concentrations in the same brain regions, as shown in a microdialysis study (unpublished observation). The increase in brain choline concentrations results in a concomitant increase in the synthesis and release of neurotransmitter acetylcholine and enhances cholinergic transmission (Ulus et al., 1978,

1989; Wecker and Schmidt, 1980; Maire and Wurtman, 1985). In addition, choline also activates nicotinic receptors as a direct agonist (Ulus et al., 1988). Thus, the observed increases in plasma ACTH and β-endorphin following intracerebroventricularly choline may result from its presynaptic effects, by increasing the synthesis and release of acetylcholine, and/or from its postsynaptic action as an agonist. The attenuation of choline's effect on plasma ACTH by hemicholinium-3 favours the presynaptic mechanism. However, since HC-3 itself also increased both ACTH and β-endorphin, our results did not allow us to draw a definite conclusion in regarding the contribution of pre- and/or post-synaptic mechanisms in the observed effects of choline.

Since choline has been used for the treatment of central cholinergic dysfunctions (for review, see Bartus et al., 1982) alone or in combination with other drugs to improve cholinergic function, it is important to know the pharmacological effects caused by pharmacological doses of choline. The present data represent the first report showing the ability of choline to affect plasma levels of ACTH and β-endorphin. Choline, by increasing plasma levels of these hormones, can affect the functions for which ACTH and β-endorphin have a regulatory role.

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