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The effect of centrally injected CDP-choline on respiratory system; involvement of phospholipase to thromboxane signaling pathway

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ABSTRACT

CDP-choline is an endogenous metabolite in phosphatidylcholine biosynthesis. Exogenous administration of CDP-choline has been shown to affect brain metabolism and to exhibit cardiovascular, neuroendocrine neuroprotective actions. On the other hand, little is known regarding its respiratory actions and/or central mechanism of its respiratory effect. Therefore the current study was designed to investigate the possible effects of centrally injected CDP-choline on respiratory system and the mediation of the central cholinergic receptors and phospholipase to thromboxane signaling pathway on CDP-choline-induced respiratory effects in anaesthetized rats.

Intracerebroventricularly (i.c.v.) administration of CDP-choline induced dose- and time-dependent increased respiratory rates, tidal volume and minute ventilation of male anaesthetized Spraque Dawley rats. İ.c.v. pretreatment with atropine failed to alter the hyperventilation responses to CDP-choline whereas mecamylamine, cholinergic nicotinic receptor antagonist, mepacrine, phospholipase A_2 inhibitor, and neomycin phospholipase C inhibitor, blocked completely the hyperventilation induced by CDP-choline. In addition, central pretreatment with furegrelate, thromboxane A_2 synthesis inhibitor, also partially blocked CDP-choline-evoked hyperventilation effects.

These data show that centrally administered CDP-choline induces hyperventilation which is mediated by activation of central nicotinic receptors and phospholipase to thromboxane signaling pathway.

1. Introduction

Respiration is a vital homeostatic neural process, controlling levels of oxygen and carbon dioxide in blood and tissues, which are crucial for life. Regulation of respiratory system involves coordinated activities of peripheral chemoreceptors and central chemosensors within the brain stem respiratory network. It is well known that cholinergic system plays an important role in the neural control of breathing (for review see Shao and Feldman, 2009). Nicotinic acetylcholine receptors (nAChRs) are expressed in brainstem and spinal cord neurons that are involved in the control of respiration (Woolf and Butcher, 1989; Woolf, 1991). These receptors mediate central cholinergic regulation of respiration and effects of the exogenous ligand nicotine on respiratory pattern (Woolf and Butcher, 1989; Woolf, 1991; Shao and Feldman, 2009). Activation of nAChRs depolarizes inspiratory neurons in PreBötzinger Complex, leading to increases in respiratory frequency (Quitadamo et al., 2005; Smith et al., 1991). These receptors are also present in motor nuclei innervating respiratory muscles. Activation of nAChRs on hypoglossal motoneurons depolarizes these neurons, potentiating tonic and respiratory-related rhythmic activity (Aldes, 1995).

Cytidine 5'-diphosphocholine (CDP-choline or citicoline) is a highly bioavailable compound with potential benefits for aiding neural repair and increasing acetylcholine levels in the central and peripheral nervous system (Adibhatla et al., 2004). CDP-choline is composed of cytidine and choline linked by a diphosphate bridge and is an essential intermediate in the synthesis of phosphatidylcholine, major brain phospholipids, via Kennedy pathway (Secades, 2011). Phosphatidylcholine is hydrolyzed by phospholipases and releases free fatty acids including arachidonic acid (AA) (Gauster et al., 2005). Exogenous CDP-choline is hydrolyzed and absorbed as cytidine and choline, and CDP-choline is resynthesized

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from cytidine triphosphate (CTP) and phosphocholine by CTP-phosphocholine cytidyltransferase (Adibhatla et al., 2004).

Our laboratory has been studying on CDP-choline for more than decade and reported lots of data on its central effects in normal and stimulated situations. Our results have shown that CDP-choline can induce pressor and bradycardic response in normal rats (Savci et al., 2002a), and cause resuscitating effects in rats subjected to hemorrhagic shock (Savci et al., 2002a, 2003). The activation of central cholinergic receptors (nicotinic and/or muscarinic) through the increase in brain acetylcholine synthesis followed by increased levels of brain choline mediate these effects (Savci et al., 2002a, 2003). CDP-choline is also able to restore the blood flow of superior mesenteric and renal arteries to prolong the survival time in anaesthetized hemorrhaged rats (Yilmaz et al., 2006), to exert strong protection against cardiac arrhythmias and to lower mortality rates in rat myocardial ischemia reperfusion model (Yilmaz et al., 2008). Moreover it has been reported that CDP-choline produced significant endocrine effects in normal and stimulated situations (Cavun and Savci, 2004; Cavun et al., 2004; Savci et al., 2002b, 2003; Savci and Wurtman, 1995). CDP-choline also may induce analgesia in rodent model of inflammatory pain by activating α7-nAChR (Bagdas et al., 2011; Gurun et al., 2009).

Central phospholipase (PL) - AA-prostaglandin (PG) signaling pathways also has an important role in the central regulation of vital systems including the cardiovascular system (Aydin and Yalcin, 2008; Yalcin and Erturk, 2007; Yalcin, 2011). Previous studies have also showed interactions between the central PL-AA-PG signaling pathways and other neuronal systems including cholinergic systems (Yalcin et al., 2005a; Yalcin et al., 2007, 2009; Yalcin and Erturk, 2007) and histaminergic system (Altinbas et al., 2012). It was previously reported that activation of cholinergic receptors by cholinergic agonists, i.e. acetylcholine, carbachol or nicotine, activates the AA cascade and that the increased generation of PGs may play a role in mediating effects of these cholinergic agents (Busija et al., 1998; De Simone et al., 2005; Orman et al., 2005; Shuttleworth and Thompson, 1998).

Considering the above data, the primary aim of the current study was to show the effect of centrally administrated CDP-choline on respiratory system and its central mechanism at least in terms of the central cholinergic and PL-AA-PG signaling pathway in anaesthetized rats.

2. Methods

2.1. Animals

Ninety male adult Sprague Dawley rats (230–280 g) were purchased from Experimental Animals Breeding and Research Center, Uludag University, Bursa, Turkey. Four-five rats were maintained with food and water *ad libitum* in individual cages under controlled conditions (20–22 °C, 60–70% humidity and 12 h light/dark light cycle). All experimental procedures were performed according to Animal Care and Use Committee of Uludag University and were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (http://oacu.od.nih.gov/regs/guide/guide1.htm).

Each animal was studied on only one occasion in a single experimental protocol and there were five rats in each experimental group.

2.2. Surgical procedures

Under the mixture of ketamine (70 mg/kg) and xylazine (10 mg/kg) anesthesia, the trachea of rats was catheterized with tracheal cannula (BS4 73-2941, Harvard Apparatus Inc., Holliston, USA) in order to monitor respiratory parameters. To deliver drug intracerebroventricularly (i.c.v.), a burr hole was drilled through the skull 1.5 mm lateral to midline and 1.0 mm posterior to bregma by using stereotaxic frame. A 22-gauge stainless steel hypodermic tubing was directed through the hole toward the lateral ventricle. The cannula was lowered 4.2 mm below the surface of the skull and was fixed to the skull by using acrylic cement. After surgery process, rats were placed in the supine position on a heated operating platform to maintain a rectal temperature of 37 ± 0.2 °C. Temperature of animals was monitored using a rectal probe throughout the study.

2.3. Respiratory parameters recording

In order to record respiratory parameters, the tracheal cannula was connected airflow head for rats (RX137, BIOPAC Systems Inc.) attached with differential pressure transducer (SS40L, BIOPAC Systems Inc.). Findings were recorded and analyzed by using the MP36 system and AcqKnowledge software (BIOPAC Systems Inc.). The tidal volume and respiratory rate of rats were obtained by the electronic airflow signal analyzing a minute's breathes. The minute ventilation of rats was calculated from tidal volume and respiratory frequency and stated milliliters in 1 min (ml/min).

2.4. Experimental protocol

In the present study, first, the dose and time relation of respiratory responses to CDP-choline were studied in 20 rats, as a main control for the study. Following baseline measurements, CDP-choline (0.5, 1 and 2 μmol) or saline (5 μL) was delivered i.c.v. and changes of respiratory parameters were recorded for the next 60 min. Doses of CDP-choline were chosen from our previous study (Savci et al., 2002a).

Second, to show mediation of the central cholinergic and prostaglandinergic signaling in the respiratory effects evoked by CDP-choline, pretreatment with a cholinergic nonselective nicotinic receptor antagonist mecamylamine (100 μg; i.c.v.), a cholinergic nonselective muscarinic receptor antagonist, atropine (10 μg; i.c.v.), PLA_2 inhibitor mepacrine (250 μg; i.c.v.), PLC inhibitor neomycin (250 μg; i.c.v.), nonselective COX inhibitor ibuprofen (200 μg; i.c.v.), or TXA_2 synthesis inhibitor furegrelate (250 μg; i.c.v.) was carried out 5 min before injection with CDP-choline (1 μmol; i.c.v.), and the values of respiratory parameters were monitored. Dose of atropine, mecamylamine (Savci et al., 2002a), mepacrine, neomycin (Yokotani et al., 2000), ibuprofen (Okuma et al., 1996) and furegrelate (Yalcin, 2011) were chosen from our and others' previous studies.

2.5. Drugs and i.c.v. injection of drugs

The following drugs were used: CDP-choline, atropine, mecamylamine mepacrine, neomycin, ibuprofen, furegrelate (Sigma-Aldrich Co. Deisenhofen, Germany). All drug solutions were prepared freshly in saline on the day of the experiment.

I.c.v. drug delivery was performed by using hand-made injection cannula (28 gauge stainless steel tubing; made by Murat Yalcin). Injection cannula was connected to a polyethylene tubing, which was filled with saline or saline containing the desired dose of the drug of interest in a 10 μl microsyringe. For the i.c.v. injection, cannula was inserted through the guide cannula, 5 μl volume of drug was infused slowly within 60 s. During the injection, an air bubble moving in the polyethylene tubing was closely watched to ensure the drug was delivered in its entirety. At the end of the experiment, animals were sacrificed by using an overdose i.v.