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The involvement of central cholinergic system in the pressor effect of intracerebroventricularly injected U-46619, a thromboxane A2 analog, in conscious normotensive rats

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Abstract The aim of this study was to determine the involvement of the central cholinergic system in the rise in blood pressure evoked by the thromboxane A2 (TxA2) analog, U-46619, given centrally. Intracerebroventricular (i.c.v.) injections of U-46619 (0.5, 1.0 and 2.0 µg) caused dose- and time-related increases in blood pressure and decreased heart rate in awake rats. U-46619 (1 µg; i.c.v.) also produced an approximately 65% increase in posterior hypothalamic extracellular acetylcholine and choline levels. Pretreatment with SQ-29548 (8 µg; i.c.v.), selective TxA2 receptor antagonist, completely inhibited both the cardiovascular responses and the increase in acetylcholine and choline levels to subsequent injection of U-46619 (1 µg; i.c.v.). Atropine (10 µg; i.c.v.), nonselective muscarinic receptor antagonist, pretreatment did not affect the cardiovascular responses observed after U-46619 (1 µg; i.c.v.). Pretreatment with the nonselective nicotinic receptor antagonist, mecamylamine (50 µg; i.c.v.) attenuated the pressor effect of U-46619 (1 µg; i.c.v.). Higher doses of mecamylamine (75 and 100 µg; i.c.v.) pretreatments did not change the magnitude of the blockade of pressor response to U-46619; however, they abolished the bradycardic effect of U-46619 dose-dependently. Interestingly, pretreatment of rats with methyllycaconitine (10 µg; i.c.v.) or α -bungarotoxin (10 µg; i.c.v.), selective antagonists of α 7 subtype of nicotinic acetylcholine receptors (α 7nAChRs), partially abolished the pressor response to i.c.v. injection of U-46619 (1 µg). Similar to the mecamylamine data, the use of higher doses of methyllycaconitine (25 and 50 µg; i.c.v.) produced the same

magnitude of blockade that was observed after the 10 µg methyllycaconitine pretreatment, but it completely abolished the bradycardic effect of U-46619 (1 µg; i.c.v.) at the dose of 25 µg. The present results show that central administration of U-46619 produces pressor and bradycardic effect and increase in hypothalamic acetylcholine and choline levels by activating central TxA2 receptors. The activation of central nicotinic receptors, predominantly α 7nAChRs, partially mediates the cardiovascular responses to i.c.v. injection of U-46619.

Keywords Thromboxane A2 · Cholinergic · Acetylcholine · Choline · Blood pressure · Nicotinic · Posterior hypothalamus

Introduction

Thromboxane A2 (TxA2) is one of the biologically active and oxygenated metabolites of prostaglandins (see Narumiya et al. 1999). It is synthesized within the central nervous system of mammals (Shohami et al. 1982; Sirko et al. 1989; Kong et al. 1991) and mRNA for its receptors are expressed in brain stem and astroglial cells of rats (Gao et al. 1997). TxA2 is one of the most potent constrictors of smooth muscles and aggregators of platelets (Narumiya et al. 1999; Bos et al. 2004). It has been suggested that it acts as a neuromediator and/or neuromodulator in the central regulation of a variety of functions including cardiovascular and neuroendocrine activities (Brooks et al. 1986; Armstead et al. 1988; Murakami et al. 1998; Tong et al. 1998). Recent studies demonstrate that centrally injected U-46619, a synthetic TxA2 analog, increases blood pressure in normal conditions (Gao et al. 1997; Wilcox et al. 1997; Yalcin and Savci 2004) and reverses hypotension in haemorrhagic shock (Yalcin and Savci 2004). These studies also implicate that cardiovascular effects of U-46619 are mediated by the activation of central TxA2 receptors.

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Recent evidence implies that pre-junctional TxA2 receptors exist on cholinergic nerve endings and that TxA2 plays an important role in the modulation of the cholinergic system in airway hyperresponsiveness in asthmatic subjects (see for review, Devillier and Bessard 1997; Spicuzza et al. 2001). Both TxA2 and U-46619 act pre-junctionally to enhance the release of acetylcholine from cholinergic nerves in canine airway smooth muscle and this prostanoid elicited bronchoconstriction partly through the activation of the cholinergic mechanism (Chung et al. 1985; Saroea et al. 1995). On the other hand, it has been suggested that eicosanoids may play a role in maintaining the sensitivity of cholinergic signalling mechanisms in the central nervous system (Buccafusco 1996) since earlier findings demonstrated that changing brain prostaglandin synthesis could affect the pressor response to cholinergic agents (Buccafusco et al. 1993).

The central cholinergic system has been repeatedly shown to play an important role in the regulation of the cardiovascular system. Centrally acting cholinergic agonists and indirectly acting cholinergic drugs have been demonstrated to increase arterial blood pressure (see for review Buccafusco 1996). Previously, we reported that choline (Arslan et al. 1991) or CDP-choline (Savci et al. 2002), considered to be a choline donor, produced an increase in blood pressure and decrease in heart rate by activating central cholinergic transmission. Taking those observations together with the previous findings about the modulation of the functions of the cholinergic system by prostaglandins including TxA2, we suggested that the cardiovascular effects of TxA2, at least in part, might be the result of the activation of central cholinergic mechanisms. In the present study, we aimed to determine the involvement of the central cholinergic system in the pressor effect of centrally injected U-46619 under normal conditions.

Materials and methods

One hundred and twenty-four adult male Sprague Dawley rats (250–300 g; Experimental Animals Breeding and Research Center, Uludag University, Bursa, Turkey) were used in the present study. Rats were housed under 12-h light/12-h dark conditions with free access to food and water. The surgical and experimental protocols were approved by the Animal Care and Use Committee of Uludag University and are in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals*.

Surgical procedures Under sevoflurane (2–4% in 100% O₂) anaesthesia, the left common carotid artery of the rats was cannulated with PE 50 tubing filled with heparinised saline (250 U/ml). For intracerebroventricular (i.c.v.) injection of drugs, a burr hole was drilled through the skull 1.5 mm lateral to the midline and 1.0 mm posterior to the bregma. A 22-gauge stainless steel hypodermic tube was directed through the hole toward the lateral ventricle. The cannula was lowered 4.5 mm below the surface of the

skull and was fixed to the skull with acrylic cement. After surgery, the rats were placed in individual cages and allowed to recover from anaesthesia for 4–5 h. During this period, the rats remained calm and showed no evidence of pain.

Blood pressure recording After the recovery period, the arterial cannula was connected to a volumetric pressure transducer (BPT 300) attached to a DA100B general purpose transducer amplifier (Commat, Ankara, Turkey). Blood pressures and heart rates were recorded and analysed using the MP100 system and AcqKnowledge software (BIOPAC Systems, Santa Barbara, CA, USA). The blood pressure is reported as mean arterial pressure (mmHg) and heart rate is expressed as beats/min.

Experimental protocol After the connection of the arterial line to the MP100 system through the transducer, the baseline blood pressure and heart rate measurements of rats were recorded. Rats were allowed to be stabilised for 20 min, then i.c.v. injections were made. Recording of cardiovascular parameters was continued for the next 60 min. In pretreated rats, SQ-29548 (8 µg), atropine (10 µg), mecamlamine (50, 75 and 100 µg), methyllycaconitine (10, 25 and 50 µg) and α -bungarotoxin (10 µg) were administered intracerebroventricularly 15 min before saline (10 µl; i.c.v.) or U-46619 (0.5, 1 and 2 µg; i.c.v.) injections. At the end of the experiments, animals were sacrificed by the intraperitoneal injection of an overdose of sodium pentothal.

All antagonist doses were chosen from previous experiments. The doses of atropine (10 µg; i.c.v.) and mecamlamine (50 µg; i.c.v.) are able to block the muscarinic and nicotinic effects of choline (150 µg; i.c.v.; Arslan et al. 1991; Ulus et al. 1995; Savci et al. 1996a,b), the dose of SQ-29548 (8 µg; i.c.v.) is able to block the pressor effect of i.c.v.-injected U-46619 (1 µg; Yalcin and Savci 2004) and the doses of the antagonists of α 7nAChR, methyllycaconitine (10 µg; i.c.v.) and α -bungarotoxin (10 µg; i.c.v.) are able to block the pressor effect of 150 µg choline injected i.c.v. (Li and Buccafusco 2004).

Microdialysis study Hand-made microdialysis probes (designed and produced by Sami Aydin) were used. Rats were anaesthetised by sevoflurane (2–4% in 100% O₂) and placed in a stereotaxic frame. The skull was exposed and drilled over the posterior hypothalamus (coordinates: 3.6 mm posterior to the bregma, 0.5 mm lateral to the midline and 9.0 mm vertical to the skull). Probes (molecular weight of the cut-off dialysis membrane was 18,000 Da and its length was 2.0 mm) were implanted and then fixed with acrylic cement to the skull. After a 24-h recovery period, a microdialysis probe was attached to a perfusion pump and an arterial catheter was connected to the transducer. The dialysis probe was perfused with artificial cerebrospinal fluid (pH 7.4) of the following composition: 120 mM NaCl, 1.3 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM NaH₂PO₄, 3.5 mM KCl,

25 mM NaHCO₃, 10 mM glucose and 20 µM neostigmine. The perfusion rate was 2 µl/min. Dialysate samples were collected at 10-min intervals. The dialysis probe was perfused with artificial cerebrospinal fluid for the first 60 min of the stabilisation period. After this period, three consecutive samples were collected and these samples were measured as the basal choline and acetylcholine levels of the rats. Then U-46619 or saline was injected i.c.v. and collection of the samples continued for 60 min. For the studies in which the TxA2 receptor antagonist was used, SQ-29548 or 5% dimethylsulfoxide (DMSO) was administered as its vehicle 15 min before the U-46619 injections.

At the end of the experiments, animals were sacrificed, and the brain was then removed and fixed in 10% formalin. Serial coronal sections measuring 40 µm were sliced in a cryostat. They were stained with haematoxylin-eosin to verify the location of the tip of the dialysis probe.

HLPC measurement of choline and acetylcholine levels Dialysate samples were injected to a high performance liquid chromatography (HPLC) system combined with an immobilised enzyme reactor and an electrochemical detector (Jasco 840 EC). Briefly, choline and acetylcholine were separated on a cation exchange column (from Bioanalytical Systems, West Lafayette, IN, USA). An enzyme reactor containing acetylcholinesterase and choline oxidase (from Bioanalytical Systems) converted acetylcholine into choline and then choline into hydrogen peroxide. Hydrogen peroxide was then electrochemically detected with a platinum electrode at +0.500 V. The mobile phase consisting of 0.05 M Na₂HPO₄ (pH 8.5) and antibacterial Kathon (International Nomenclature Cosmetic Ingredient [INCI] name: methylchloroisothiazolinone and methylisothiazolinone; from Bioanalytical Systems; 0.5%) was delivered by an HPLC pump (Model LC-9A; Shimadzu, Columbia, MD, USA). The flow rate was 1.0 ml/min. Chromatograms were completed within 6 min.

Drugs The following drugs were used: U-46619 (Calbiochem, EMD Biosciences, Darmstadt, Germany), SQ-29548 (Cayman Chemical, Ann Arbor, MI, USA), atropine sulfate, mecamlamine HCl, methyllycaconitine and α -bungarotoxin (Sigma-Aldrich Chemie, Schnellendorf, Germany). All drugs except U-46619 and SQ-29548 were dissolved in saline (0.9% NaCl). U-46619 was shipped in methyl acetate and stored at -50°C. Stock solutions were prepared by the addition of an equal volume of Tris buffer (0.1 M), dried in a stream of air and stored at -50°C. The dilutions were made freshly in saline before each experiment. SQ-29548 was dissolved in saline containing 5% DMSO. All doses of drugs refer to the free base.

None of the drugs used throughout the study caused any overt behavioural effects.

Intracerebroventricular injection of drugs For i.c.v. injections, 50 µl Hamilton microsyringe was connected to an injection cannula (28-gauge stainless steel tubing) through the polyethylene tubing, which was filled with saline or

saline containing the desired dose of the drug of interest. In the SQ-29548 pretreated group, the 5% DMSO in saline solution was used as a control in separate animals. The injection cannula was inserted into the guide cannula (22-gauge stainless steel tubing). Drugs at a volume of 10 µl were then delivered by hand slowly within 40–60 s. At the end of each experiment, the injection site was verified by injecting 10 µl of India ink.

Data and statistical analysis Data are represented as mean \pm standard error of the mean (SEM). Repeated measures of variance analysis (RM-ANOVA) (two-way) was performed for the appropriate groups. Dunnett's test was performed as an *a posteriori* test when significant interactions were found. A *P* value of <0.05 was considered significant.

Results

Cardiovascular effects of U-46619 in normotensive rats

Although we reported previously that in normal conditions, i.c.v.-injected U-46619 increases blood pressure of awake rats (Yalcin and Savci 2004), we repeated those experiments here since they are the main control group of this study. Intracerebroventricular injection of U-46619 (0.5, 1.0 and 2.0 µg) produced a prompt and dose-related increase in blood pressure (Fig. 1; top). Increases in blood pressure were 11 \pm 3 mmHg (*n*=8) and 22 \pm 4 mmHg (*n*=8) after the injection of 0.5 µg and 1.0 µg U-46619 respectively. The magnitude of the increase in blood pressure after the injection of 2.0 µg U-46619 was similar, but slightly longer lasting than that observed in rats injected with 1.0 µg. The pressor effect of 0.5–2.0 µg U-46619 reached its maximum within 1–5 min and returned back to control values 10–15 min after the injection. Analysis of variance confirmed that U-46619 administration produced a significant dose, $F(3,27)=13.50$, $P<0.001$, and time, $F(10,297)=4.53$, $P<0.001$, and an insignificant dose–time interaction on blood pressure effect, $F(30,297)=0.69$, $P>0.05$.

U-46619 injection caused the decrease in heart rate in a dose-dependent manner (dose, $F(3,27)=21.27$, $P<0.001$, time, $F(10,297)=1.21$, $P>0.05$), dose–time, $F(30,297)=0.47$, $P>0.05$; Fig. 1; bottom). The maximum decrease in heart rate was 37 \pm 7 beats/min and it was observed within 3–5 min of the injection of 2 µg U-46619. The bradycardic effect lasted till 10–15 min after the injection of 0.5–2.0 µg U-46619 (Fig. 1; bottom).

Effect of U-46619 administration on the extracellular acetylcholine and choline levels in posterior hypothalamus

In order to determine if U-46619 is able to affect the extracellular levels of acetylcholine and choline when given at the dose where it produced pressor effect, we performed

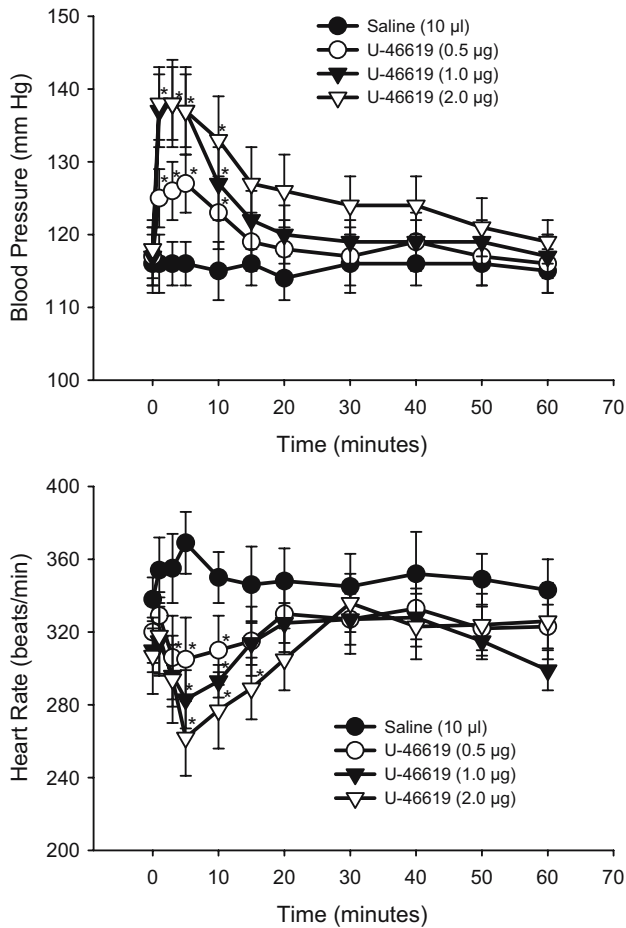


Fig. 1 Effect of intracerebroventricularly (i.c.v.) injected U-46619 on blood pressure (*top*) and heart rate (*bottom*) of rats under normal conditions. U-46619 (0.5, 1.0 and 2.0 µg; i.c.v.) or saline (10 µl; i.c.v.) were administered and cardiovascular parameters were monitored for 60 min. Data are given as mean±SEM of seven to eight measurements. Statistical analysis was performed using the two-way repeated measures of variance analysis (RM-ANOVA) with a *posthoc* Dunnett's test. * $P < 0.05$ was considered significantly different from the value of the saline group

a microdialysis study in the posterior hypothalamic area, which is very important for the cardiovascular regulation. In this study, basal acetylcholine and choline levels were 0.41 ± 0.02 pmol/10 min ($n=10$) and 3.05 ± 0.1 pmol/10 min ($n=10$) respectively. U-46619 injection (1 µg; i.c.v.) produced an approximately 65% increase in hypothalamic acetylcholine (dose, $F(1,8)=22.8$, $P < 0.001$, time, $F(6,48)=15.8$, $P < 0.001$, and dose-time, $F(6,48)=3.4$, $P < 0.01$, interaction; Fig. 2; top) and choline levels (dose, $F(1,8)=8.1$, $P < 0.05$, time, $F(6,48)=5.0$, $P < 0.001$, and dose-time, $F(6,48)=1.6$, $P > 0.05$, interaction; Fig. 2; bottom).

Effect of SQ-29548 pretreatment on the cardiovascular responses and increase in acetylcholine and choline levels to i.c.v.-injected U-46619 in normotensive rats

We next aimed to determine whether central TxA2 receptor activation mediated the pressor and bradycardic effect of

U-46619 together with the increase in hypothalamic acetylcholine and choline levels in the present study. SQ-29548, a TxA2 receptor antagonist, (8 µg; i.c.v.) or its vehicle (5% DMSO; 10 µl) was injected 15 min before the U-46619 (1 µg; i.c.v.) administration. Baseline blood pressure and heart rate values of SQ-29548 or vehicle-injected rats were 122 ± 3 mmHg and 324 ± 11 beats/min or 119 ± 2 mmHg and 335 ± 11 beats/min respectively. These values did not significantly ($P > 0.05$) change after vehicle or SQ-29548 injection (15 min after injection values in SQ-29548 or vehicle-injected rats were 121 ± 2 mmHg, 338 ± 11 beats/min or 121 ± 2 mmHg, 322 ± 10 beats/min respectively). SQ-29548 pretreatment completely blocked the blood pressure (Fig. 3; top) and heart rate (Fig. 3; bottom) responses to U-46619. Also, i.c.v. injection of vehicle or SQ-29548 did not affect hypothalamic extracellular acetylcholine and choline levels of rats (for acetylcholine levels before vehicle: 0.29 ± 0.02 pmol/10 min and after vehicle: 0.30 ± 0.02 pmol/10 min, before SQ-29548: $0.39 \pm$

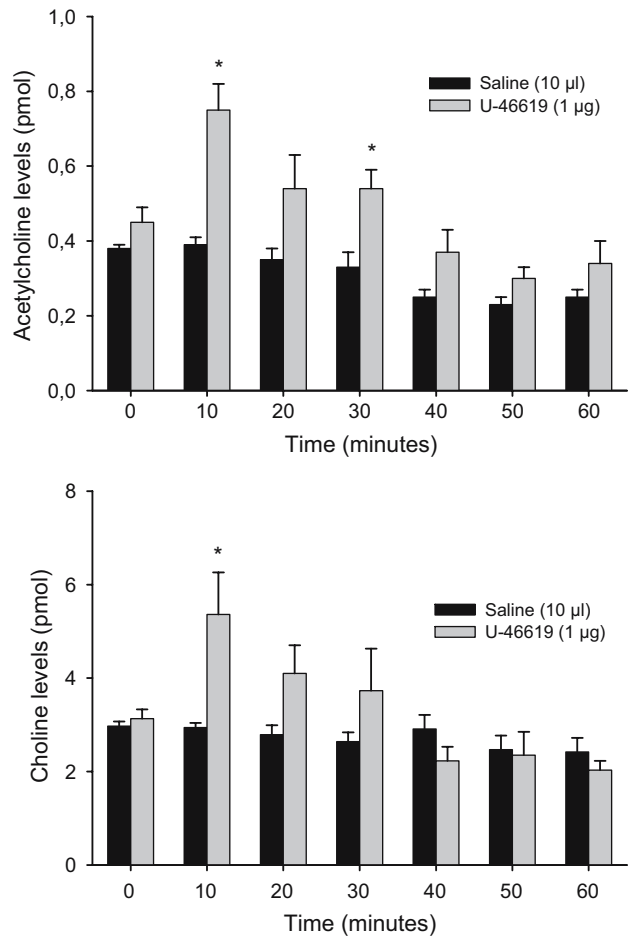


Fig. 2 Effect of U-46619 on extracellular acetylcholine and choline levels in the posterior hypothalamic area. After the collection of basal samples, rats were administered U-46619 (1.0 µg; i.c.v.) or saline (10 µl; i.c.v.) and samples continued to be collected at 10-min intervals. Data represent the mean±SEM of five measurements. Statistical analysis was performed using a two-way RM-ANOVA with a *posthoc* Dunnett's test. * $P < 0.05$ was considered significantly different from the value of the saline group

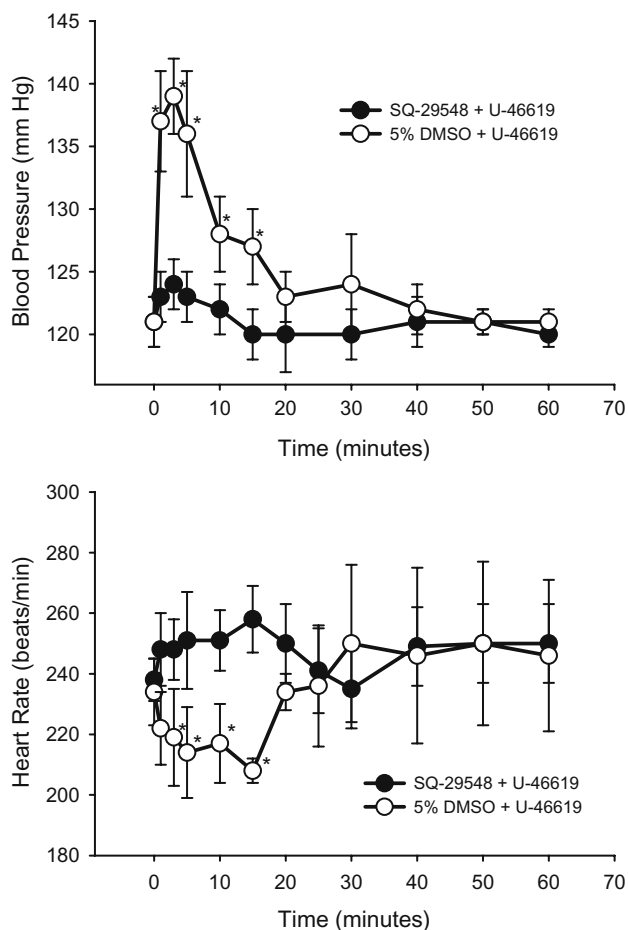


Fig. 3 Effect of SQ-29548 pretreatment on blood pressure (*top*) and heart rate (*bottom*) responses to i.c.v.-injected U-46619. SQ-29548 (8 μ g; i.c.v.) or vehicle (5% DMSO 10 μ l; i.c.v.) was administered 15 min before U-46619 (1.0 μ g; i.c.v.) injection. Cardiovascular parameters were monitored for 60 min. "0" represents the time point at which U-46619 was injected. Data are given as mean \pm SEM of five measurements. Statistical analysis was performed using a two-way RM-ANOVA with a *posthoc* Dunnett's test. * P <0.05 was considered significantly different from the value of the control group

0.05 pmol/10 min and after SQ-29548: 0.37 ± 0.05 pmol/10 min; for choline levels before vehicle: 3.6 ± 0.4 pmol/10 min and after vehicle: 3.6 ± 0.4 pmol/10 min, before SQ-29548: 3.8 ± 0.2 pmol/10 min and after SQ-29548: 4.2 ± 0.2 pmol/10 min). SQ-29548 pretreatment also abolished both increases in acetylcholine (dose, $F(1,8)=12.7$, P <0.05, time, $F(7,56)=15.7$, P <0.001, dose-time, $F(7,56)=7.3$, P <0.001) and choline (dose, $F(1,8)=7.8$, P <0.05, time, $F(7,56)=9.4$, P <0.001, dose-time, $F(7,56)=3.9$, P <0.01) levels in the hypothalamic area after U-46619 (Fig. 4).

Effects of atropine or mecamylamine pretreatment on the cardiovascular effects of U-46619 under normal conditions

We subsequently determined the involvement of central cholinergic receptors in the pressor and bradycardic effect

of U-46619. The nonselective muscarinic receptor antagonist, atropine (10 μ g; i.c.v.) or nonselective nicotinic receptor antagonist, mecamylamine (50, 75 and 100 μ g; i.c.v.) pretreatment was performed 15 min before the injection of U-46619 (1 μ g; i.c.v.). Baseline values of blood pressure and heart rates of the rats were shown in Table 1. Neither atropine nor mecamylamine administration changed those baseline values at the end of the 15-min period. Atropine pretreatment did not affect blood pressure and heart rate responses to U-46619 (1 μ g; i.c.v.; Fig. 5; top and bottom). However, the pressor effect of U-46619 was greatly attenuated by the mecamylamine pretreatment (Fig. 6). The degree of the attenuation in the pressor response was similar at all doses of mecamylamine used (dose, $F(3,17)=5.5$, P <0.01, and time, $F(9,153)=9.4$, P <0.001, interaction). The bradycardic response to U-

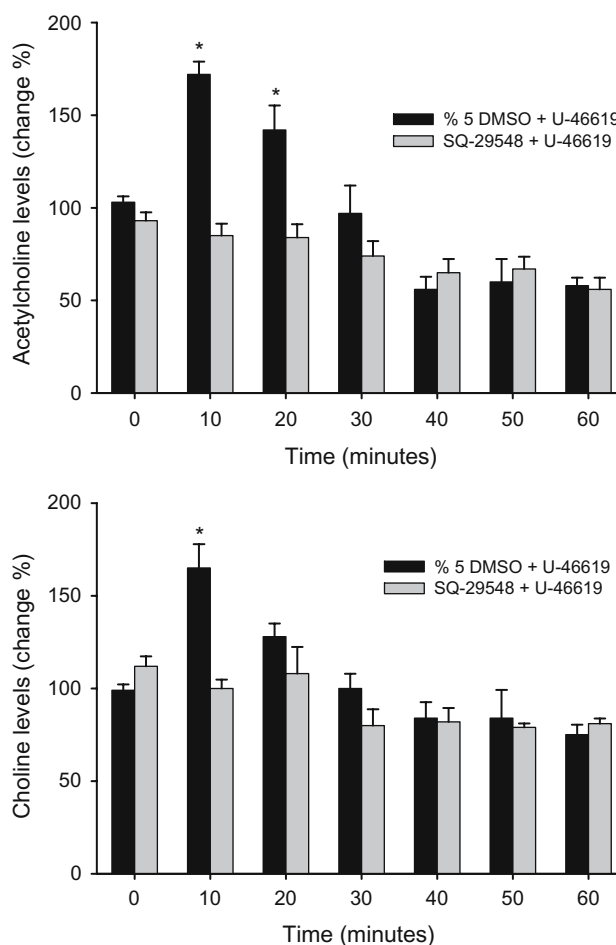


Fig. 4 Effect of SQ-29548 on the U-46619-induced increase in extracellular acetylcholine and choline levels in the posterior hypothalamic area. Rats were prepared for microdialysis experiments. After a recovery period, samples were collected at 10-min intervals. After collection of basal samples, vehicle (5% DMSO) (10 μ l; i.c.v.) or SQ-29548 (8 μ g; i.c.v.) was injected 15 min before U-46619 (1 μ g; i.c.v.) administration. Collection of samples was continued for 60 min after U-46619 injection. Data are given as mean \pm SEM of five measurements. Statistical analysis was performed using a two-way RM-ANOVA with a *posthoc* Dunnett's test. * P <0.05 was considered significantly different from the value of the saline after pretreatment group

Table 1 Baseline values of blood pressure and heart rate of each cholinergic antagonist-pretreated group

Treatment	Blood pressure (mmHg)	Heart rate (beats/min)
Saline + saline	116±2	326±24
Saline + U-46619	117±3	325±10
Atropine + U-46619	112±4	331±14
Mecamylamine + U-46619		
50 µg	114±3	338±9
75 µg	118±3	316±11
100 µg	118±3	315±18
α-bungarotoxin + U-46619	113±4	331±30
Methyllycaconitine + U-46619		
10 µg	122±3	356±8
25 µg	118±2	328±22
50 µg	119±3	319±20

Blood pressure and heart rate values represent the recordings obtained just before the first intracerebroventricular (i.c.v.) injections. Data are given as mean±SEM of four to seven measurements

46619 was completely blocked with the 100 µg of mecamylamine pretreatment, $F(3,17)=6.2$, $P<0.001$ (Fig. 6; bottom), although time and dose–time effects were not significant.

Effects of α-bungarotoxin and methyllycaconitine pretreatments on the cardiovascular effects of U-46619

Later we determined the role of the α7 subtype of nicotinic acetylcholine receptors (α7nAChRs) in the pressor response induced by U-46619. Rats were pretreated with α-bungarotoxin (10 µg; i.c.v.) or methyllycaconitine (10, 25 and 50 µg; i.c.v.), selective antagonists of α7nAChRs, 15 min before U-46619 (1 µg; i.c.v.). Table 1 shows baseline blood pressure and heart rate values of the rats. These values did not significantly change ($P>0.05$) after administration of α-bungarotoxin or methyllycaconitine. Both pretreatments at the dose of 10 µg partially blocked the increase in blood pressure induced by U-46619 (Figs. 7, 8; top). The injection of the higher doses of methyllycaconitine (25 and 50 µg) produced a similar magnitude of blockade to the pressor effect of U-46619 (Fig. 8; top). Analysis of variance indicated the significant dose and time, but not dose–time, interactive effects of methyllycaconitine (dose, $F(3,18)=7.3$, $P<0.05$, time, $F(9,162)=14.1$, $P<0.001$) on the pressor effect. The bradycardic effect of the drug was blocked by both α-bungarotoxin (Fig. 7; bottom), at a dose of 10 µg, and methyllycaconitine, at doses of 25 and 50 µg, pretreatments (dose, $F(3,18)=8.5$, $P<0.001$, time, $F(9,162)=12.8$, $P<0.001$, dose–time, $F(27, 162)=3.9$, $P<0.001$; Fig. 8; bottom). Moreover, the combination of the mecamylamine plus α-bungarotoxin or mecamylamine plus methyllycaconitine failed to attenuate

the pressor effect further than that observed with mecamylamine alone (data not shown).

Discussion

These data show that i.c.v.-injected U-46619 increased blood pressure and decreased heart rate in normotensive rats. Central administration of U-46619 also increased hypothalamic extracellular acetylcholine and choline levels. SQ-29548 pretreatment completely blocked both the cardiovascular effects and the increase in acetylcholine and choline levels in response to TxA2 analog. Atropine pretreatment failed to change the cardiovascular responses to U-46619. Mecamylamine pretreatment partially abolished the pressor effect of U-46619 and at the highest dose completely blocked the bradycardic response to the drug. Moreover, methyllycaconitine or α-bungarotoxin pretreat-

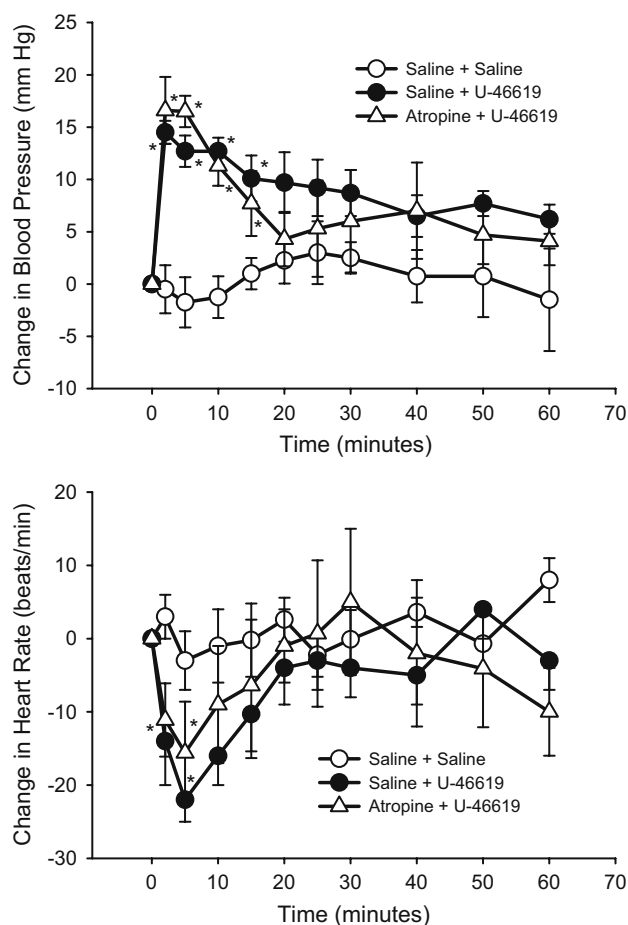


Fig. 5 Effect of atropine pretreatment on blood pressure (top) and heart rate (bottom) responses to U-46619. Atropine (10 µg; i.c.v.) or saline (10 µl; i.c.v.) was administered 15 min before U-46619 (1.0 µg; i.c.v.) or saline (10 µl; i.c.v.) injection. Cardiovascular parameters were monitored for 60 min. Data are given as mean±SEM of four to seven measurements. Statistical analysis was performed using a two-way RM-ANOVA with a *posthoc* Dunnett's test. * $P<0.05$ was considered significantly different from the value of the saline-pretreated group

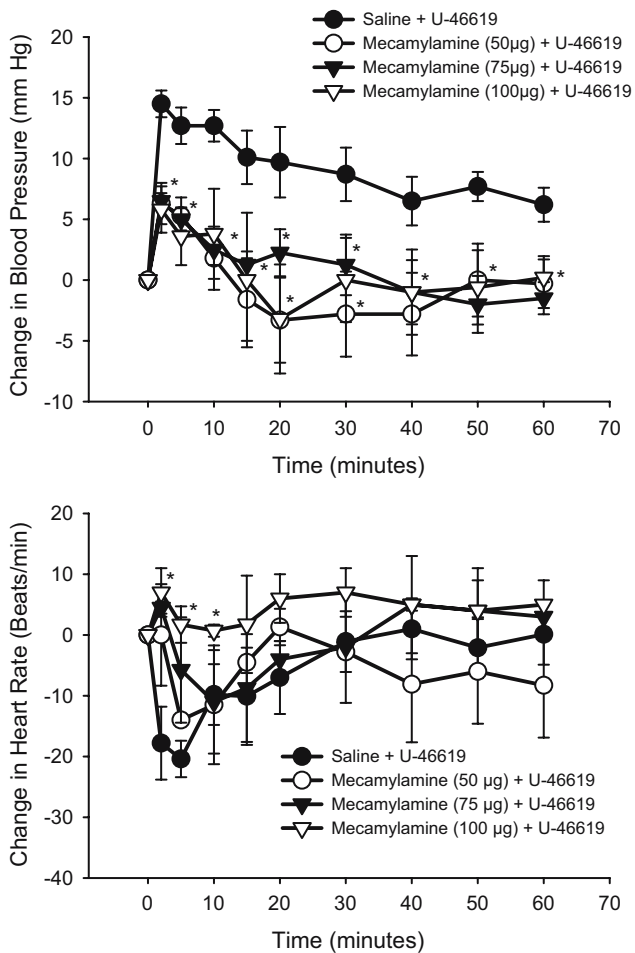


Fig. 6 Effect of mecamlamine pretreatment on blood pressure (*top*) and heart rate (*bottom*) responses to U-46619. Mecamlamine (50, 75 and 100 µg; i.c.v.) or saline (10 µl; i.c.v.) was administered 15 min before U-46619 (1.0 µg; i.c.v.) injection. Cardiovascular parameters were monitored for 60 min. Data are given as mean±SEM of four to seven measurements. Statistical analysis was performed by using a two-way RM-ANOVA with a *posthoc* Dunnett's test. * $P < 0.05$ was considered significantly different from the value of the saline-pretreated group

ments attenuated the blood pressure response to U-46619 and blocked the bradycardia induced by the drug.

The pressor and bradycardic effects of U-46619 were prompt and short-lasting. An approximately 10–20 mmHg increase in blood pressure and 40–60 beats/min decrease in heart rate were observed after i.c.v. injection of U-46619. These results show great correspondence with our previous report (Yalcin and Savci 2004). Furthermore, in accordance with previous papers reporting that central TxA2 receptors are involved in the pressor effect of U-46619 (Gao et al. 1997; Wilcox et al. 1997), we demonstrated once again that the activation of brain TxA2 receptors mediate the pressor and bradycardic effect of U-46619 since SQ-29548 pretreatment completely abolished both responses observed after injection of the drug.

The present results imply that the activation of central cholinergic mechanisms partially mediated the pressor effect of i.c.v.-injected U-46619 because:

1. Hypothalamic extracellular acetylcholine and choline levels increased after U-46619 administration
2. The blockade of central nicotinic receptors by i.c.v. mecamlamine pretreatment attenuated the pressor effect of i.c.v. U-46619
3. The blockade of the central $\alpha 7$ subtype of nicotinic AChRs by i.c.v. methyllycaconitine or α -bungarotoxin pretreatment partially abolished the increase in blood pressure observed after U-46619

To date, several studies have reported on the modulation of cholinergic transmission by prostaglandins (O'Byrne and Fuller 1989; Buccafusco et al. 1993; Saroea et al. 1995; Spicuzza et al. 2001). However, most of those studies investigated the mechanism of TxA2 in airway hyperresponsiveness and, therefore, they only imply the peripheral cholinergic modulation by TxA2 or U-46619. Besides, the results of those studies are highly variable. It was pre-

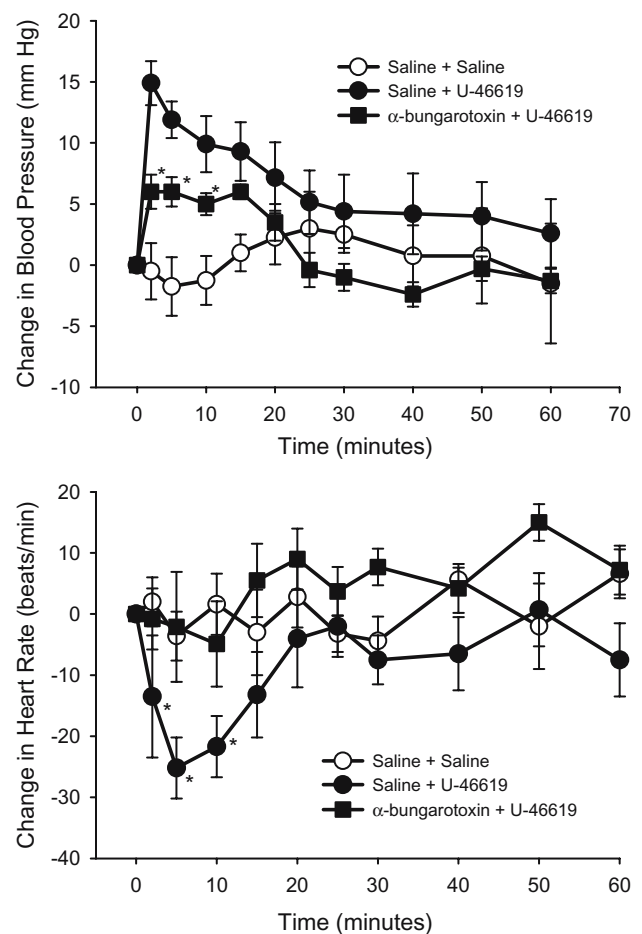


Fig. 7 Effect of α -bungarotoxin pretreatment on blood pressure (*top*) and heart rate (*bottom*) responses to U-46619. α -Bungarotoxin (10 µg; i.c.v.) or saline (10 µl; i.c.v.) was administered 15 min before U-46619 (1.0 µg; i.c.v.) or saline (10 µl; i.c.v.) injection. Blood pressure and heart rates of the rats were monitored for 60 min. Data are given as mean±SEM of four to seven measurements. Statistical analysis was performed using a two-way RM-ANOVA with a *posthoc* Dunnett's test. * $P < 0.05$ was considered significantly different from the value of the saline-pretreated group

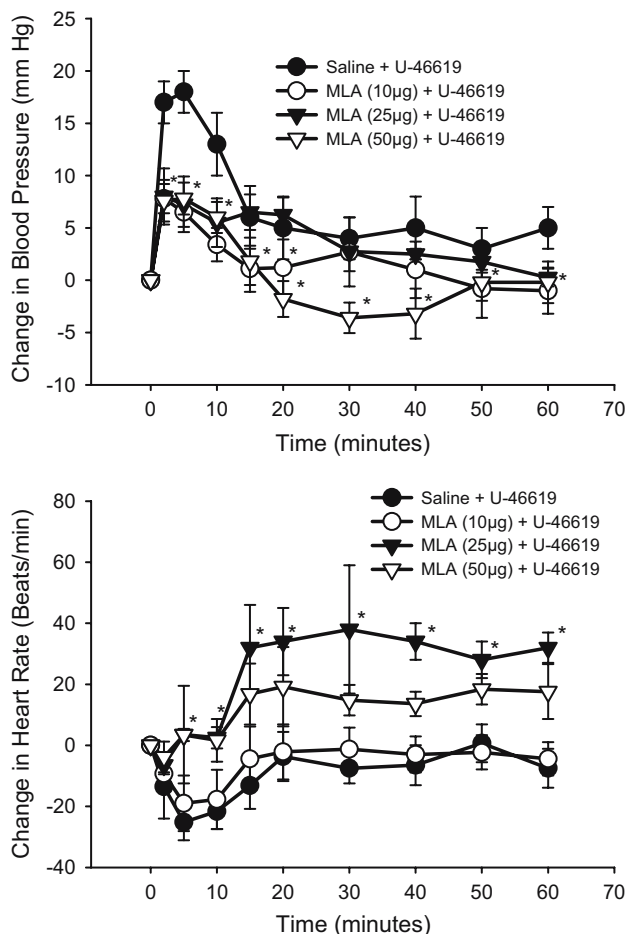


Fig. 8 Effect of methyllycaconitine pretreatment on blood pressure (*top*) and heart rate (*bottom*) responses to U-46619. Methyllycaconitine (10, 25 and 50 µg; i.c.v.) or saline (10 µl; i.c.v.) was administered 15 min before U-46619 (1.0 µg; i.c.v.) injection. Blood pressure and heart rates of the rats were monitored for 60 min. Data are given as mean±SEM of four to seven measurements. Statistical analysis was performed using a two-way RM-ANOVA with a *posthoc* Dunnett's test. * $P < 0.05$ was considered significantly different from the value of the saline-pretreated group

viously shown that both TxA₂- and U-46619-induced bronchoconstriction is partially mediated by acetylcholine release (Chung et al. 1985; Saroea et al. 1995). However, recent papers imply that in guinea pig trachea, pre-junctional TxA₂ receptors exist on cholinergic nerve endings and that the activation of these receptors by selective agonists inhibits cholinergic neurotransmission (Spicuzza et al. 2001). There is another study that demonstrated the modulation of blood pressure responses to cholinergic agents by inhibiting the brain prostaglandin synthesis (Buccafusco et al. 1993). It was performed using the non-specific cyclooxygenase inhibitor, indomethacin. Hence, our results are the first both in terms of reporting the central modulation of the cholinergic system by TxA₂ and in terms of showing for the first time that centrally injected U-46619 increases hypothalamic extracellular acetylcholine and choline levels. The increase in acetylcholine or choline levels was approximately 65% of their control values and time dependent. Microdialysing rats enabled us to see with

great ease the online time scale of the effect along with the cardiovascular parameters. The maximum acetylcholine increases were obtained within the first 10 min of injection during which the pressor and bradycardic responses to U-46619 reached their maximum values. The effects appear to be mediated by the activation of central TxA₂ receptors since the SQ-29548 pretreatment completely blocked the increase in acetylcholine and choline levels in response to U-46619. The released acetylcholine does not seem to contribute to the choline increases in the extracellular space because acetylcholinesterase was inhibited by neostigmine. This observed increase in choline levels might be the result of the U-46619-induced phospholipid breakdown, decreased uptake of this molecule to the cholinergic terminals or the increased release of choline from the nerve terminals as it seems quite possible by the observations that cholinergic terminals can store and release choline under some circumstances (Welner and Colier 1985; Klein et al. 2002; Bravo et al. 2004). However, this point of the study needs to be determined by further investigations.

The activation of central cholinergic nicotinic receptors are involved in the pressor effect of U-46619, since mecamylamine pretreatment attenuated the response while atropine failed to change the effect. The nicotinic receptor involvement is in agreement with our previous studies, which demonstrated the central nicotinic involvement in the pressor effect of choline (Arslan et al. 1991; Ulus et al. 1995) and CDP-choline (Savci et al. 2002, 2003). In recent years, many subtypes of neuronal nicotinic acetylcholine receptors, which can be constructed from different subunit combinations, have been shown in the mammalian brain (Alkondon and Albuquerque 1993; Sargent 1993; Jin et al. 2004). The $\alpha 7$ homo-oligomeric receptor is the unique member of this family, because this is the most abundant form in cultured neurons (Alkondon and Albuquerque 1993) and choline is a full and selective agonist of this receptor (Alkondon et al. 1997). It is highly expressed in hypothalamic regions as α -bungarotoxin binding sites (Gattu et al. 1997) and has been shown to mediate several cholinergic responses including cardiovascular (Li and Buccafusco 2004) and cognitive improvement (Arendash et al. 1995). In the present study, it is likely that the cholinergic nicotinic part of the pressor effect would be the result of the whole participation of this subtype of the receptor since both methyllycaconitine and α -bungarotoxin produced blockade in the pressor effect of the drug at the same extent as those seen in mecamylamine-pretreated groups. Also, the data show that the involvement of the central nicotinic receptors in the pressor effect of U-46619 is partial since the increase in the dose of mecamylamine or methyllycaconitine did not change the magnitude of the blockade. Finally, based on the reports implying that mecamylamine is a relatively specific antagonist for nAChRs containing $\alpha 3\beta 4$ subunits (Webster et al. 1999; Aberger et al. 2001), we suggested that it might be possible to produce complete blockade in the pressor effect if we use the combination of mecamylamine plus one of the $\alpha 7$ -nAChRs antagonists, methyllycaconitine or α -bungarotoxin. However, the observation of the same degree of

attenuation in the pressor response in rat pretreated by a combination of the antagonists ruled out this possibility.

The decrease in heart rate observed after U-46619 administration appears to be mediated by the activation of central TxA₂ receptors since SQ-29548 pretreatment completely blocked the response. Interestingly, central cholinergic nicotinic mechanisms seem to be involved in the bradycardic effect because both mecamlamine and α 7-nAChRs antagonists, methyllycaconitine or α -bungarotoxin, completely blocked the effect. Mecamlamine or methyllycaconitine pretreatments completely abolished the change in heart rate to U-46619 only at higher doses than those that attenuated pressor response; whereas α -bungarotoxin blocked the bradycardia at the dose that abolished the pressor response. The differential dose effect may be due to the differences in drug distribution and effectiveness in the pathway mediating the bradycardic response to U-46619.

In conclusion, i.c.v. administration of U-46619 exerts cardiovascular effects and increases hypothalamic acetylcholine and choline levels by activating central TxA₂ receptors. The activation of the central nicotinic cholinergic receptors, predominantly α 7nAChRs, mediates the cardiovascular responses to the i.c.v. injection of U-46619.

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