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The role of brain acetylcholine in $GABA_A$ receptor antagonist-induced blood-pressure changes in rat

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Abstract

Previous experimental studies have shown that intracerebroventricular (i.c.v.) injection of the $GABA_A$ receptor antagonist, bicuculline methiodide, results in marked increases in blood pressure due to an increase in sympathetic nervous system activity. It is well recognized that the central cholinergic system is also involved in the regulation of blood pressure. In the present study, we examined the role of brain acetylcholine in the pressor response induced by bicuculline methiodide in conscious Sprague-Dawley rats. I.c.v. (0.05, 0.3 and 0.5 nmol) and intrahypothalamic (40 pmol) administration of bicuculline methiodide produced blood-pressure increases in a dose-dependent manner. Hemicholinium-3 was given i.c.v. 1 h prior to bicuculline methiodide. The depletion of brain acetylcholine was demonstrated by the suppression of physostigmine-induced pressor responses, but blood pressure increases in response to carbachol remained unchanged. The pressor responses to bicuculline methiodide in animals pre-treated with hemicholinium-3 were significantly higher than those seen in saline-pre-treated groups. Likewise, bicuculline methiodide, at a dose that did not alter blood pressure alone, caused pressor responses in rats pre-treated with the nicotinic receptor antagonist, mecamylamine, whereas the muscarinic receptor antagonist, atropine, was ineffective in this respect. In conclusion, it seems likely that endogenous brain acetylcholine has a modulator role on $GABA_A$ receptor-mediated blood-pressure control via nicotinic receptors.

Keywords: $GABA_A$ receptor; Acetylcholine; Blood pressure; (Rat)

1. Introduction

γ-Aminobutyric acid (GABA) is known to be involved in central cardiovascular regulation. Drugs that interfere with the function of GABA cause cardiovascular, respiratory and behavioral changes in different species (Di Micco and Gillis, 1979; Di Scala et al., 1984; Waldrop et al., 1988). Previous experimental studies have shown that intracerebroventricular (i.c.v.) injection of the $GABA_A$ receptor antagonist bicuculline methiodide results in marked blood-pressure and heart-rate elevations due to an increase in sympathetic nervous system activity. A growing body of evidence suggests that a forebrain periventricular GABAergic system exerts a tonic inhibitory influence over the sympathetic nervous system (Di Micco and Abshire, 1987; Williford et al., 1979). Recently, studies have more directly implicated the presence of a GABAergic site located within the hypothalamus (Wible et al., 1988). Both GABA and its synthesizing enzyme, glutamic acid decarboxylase, have been demonstrated to be present in high concentrations within the hypothalamus.

It is well recognized that central cholinergic neurons are also among the most important components in the regulation of blood pressure. Stimulation of central cholinergic receptors increases arterial blood pressure in rats, humans and several other species by increasing sympathetic nerve outflow to the peripheral resistance vessels (Brezenoff and Giuliano, 1982; Özkutlu et al., 1993). In addition, cholinergic neurons in the central nervous system might be involved in the maintenance of elevated blood pressure in some experimental models of hypertension, such as spontaneously hypertensive rats (SHR) and desoxycorticosterone acetate (DOCA)-salt hypertension in rats (Giuliano and Brezenoff, 1987). Excessive central cholinergic tone was indicated by the acute hypotensive effects of brain acetycholine depletion with hemicholinium-3 or central muscarinic receptor blockade in SHR (Brezenoff and Caputi, 1980; Caputi et al., 1980; Vargas and Brezenoff,

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1988). Brain regions that evoke a pressor response upon muscarinic cholinergic stimulation include the dorsal hypothalamus, ventrolateral medulla and lateral septum. The hypothesis that the posterior hypothalamus is a primary cholinergic site involved in hypertension was supported by Brezenoff and Xiao (1989). Likewise, several studies have indicated the existence of a GABAergic inhibitory mechanism within the dorsal hypothalamus in the modulation of the cardiovascular system. The dorsomedial hypothalamic nucleus in the posterior region of hypothalamus was found to be the most effective site in $GABA_A$ receptor antagonist-induced cardiovascular responses (De Novellis et al., 1995; Gören et al., 1996).

Anatomical evidence for co-distribution of GABA- and acetylcholine-synthesizing neurons in the basal forebrain of the rat, GABAergic modulation of striatal cholinergic interneurons and an extensive neuronal connectivity of hippocampal muscarinic cholinoceptive non-pyramidal cells and the GABAergic system have been reported by several investigators (Gritti et al., 1993; De Boer and Westerink, 1994; Van Der Zee and Luiten, 1993). In contrast to the anatomical and electrophysiological characterization of the GABA-cholinergic interaction, its significance in central cardiovascular regulation remains unsettled (Dudchenko and Sarter, 1991). The purpose of the present study was to determine the role of brain acetylcholine in the pressor response induced by the blockade of central $GABA_A$ receptors with bicuculline methiodide in conscious rats.

2. Materials and methods

Female Sprague-Dawley rats weighing 200–250 g were allowed a standard laboratory rat chow and tap water ad libitum and were housed in Plexiglass cages in a temperature-controlled room (20 ± 3°C).

2.1. Cannula placement

The animals were anesthetized with ketamine (100 mg/kg i.p.) and chlorpromazine (1 mg/kg i.p.) 3–7 days before the experiments. The head of the animal was placed in a stereotaxic apparatus (Stoelting Model 51600). The scalp was longitudinally incised, and skull was leveled between lambda and bregma. A stainless steel guide cannula (Plastic Products, Roanoke, VA, USA) was implanted into the left dorsomedial hypothalamic nucleus (3.2 mm caudal and 0.5 mm lateral to bregma and 7.5 mm ventral to the surface of the skull) and/or the right lateral cerebral ventricle (1.0 mm caudal and 1.5 mm lateral to bregma and 3.2 mm ventral to the surface of the skull) on the basis of the stereotaxic atlas of Paxinos and Watson (1986). The guide cannula was fixed by dental cement together with three screws driven into the skull, and plugged with a removable stylet except during drug injection.

2.2. Study design

On the day of the experiment, a polyethylene catheter (PE-10 fused to PE-50) filled with heparinized saline was inserted into the iliac artery and routed subcutaneously to exit at the back of the neck under light ether anesthesia. An injection stylet extending 1 mm below the tips of the guide cannulas was placed for i.c.v. administration of the vehicle or test compounds. The animal was placed in a Plexiglas cage and allowed to rest quietly for 2–4 h prior to the experiment. The extension tubing of the iliac catheter was attached to a pressor transducer and blood pressure was recorded on a polygraph (Grass Model 7, USA). Following the stabilization period, basal blood pressure was measured. Then, drugs were administered either by i.c.v. or intraparanchymal routes in a volume of 10 μl or 200 nl, respectively. Injections were given slowly within 30 s via a Hamilton microsyringe through the extension tubing so that animal was not handled during the test period.

The effect of the reduced brain acetylcholine levels on central cholinergic function was determined by comparing the pressor response to direct and indirect central cholinergic stimulation. For this purpose, rats were injected with carbachol or physostigmine 1 h after hemicholinium-3 injection in the first series of experiments. Hemicholinium-3 was administered i.c.v. at a dose of 9 nmol (5 μg), since this quantity previously has been shown to cause maximum depletion of brain acetylcholine (Brezenoff and Rusin, 1974). In the second series of experiments, the animals were injected with bicuculline methiodide i.c.v. (0.05, 0.3 and 0.5 nmol) or into the dorsomedial hypothalamic nucleus (40 pmol) 1 h after the administration of hemicholinium-3. In the last series of experiments, a dose of 245 nmol (50 μg) of mecamylamine hydrochloride or 15 nmol (10 μg) of atropine sulfate was given i.c.v. 30 min before the injection of 0.05 nmol of bicuculline methiodide. Saline (10 μl) was given to the control rats instead of hemicholinium-3, mecamylamine or atropine.

2.3. Histological examination

For the verification of injection site, the animals were anesthetized with urethane (1.2 mg/kg i.p.) and perfused transcardially with saline followed by 4% buffered formalin solution, after each experiment. Methylene blue was injected in a volume of 200 nl into the dorsomedial hypothalamic nucleus and/or 10 μl into the lateral ventricle. The brains were then removed and kept in a 20% sucrose-formalin solution for 1 week. 40 μm coronal sections were cut through the dorsomedial hypothalamic nucleus region by using a cryostat (Microm, Germany) and

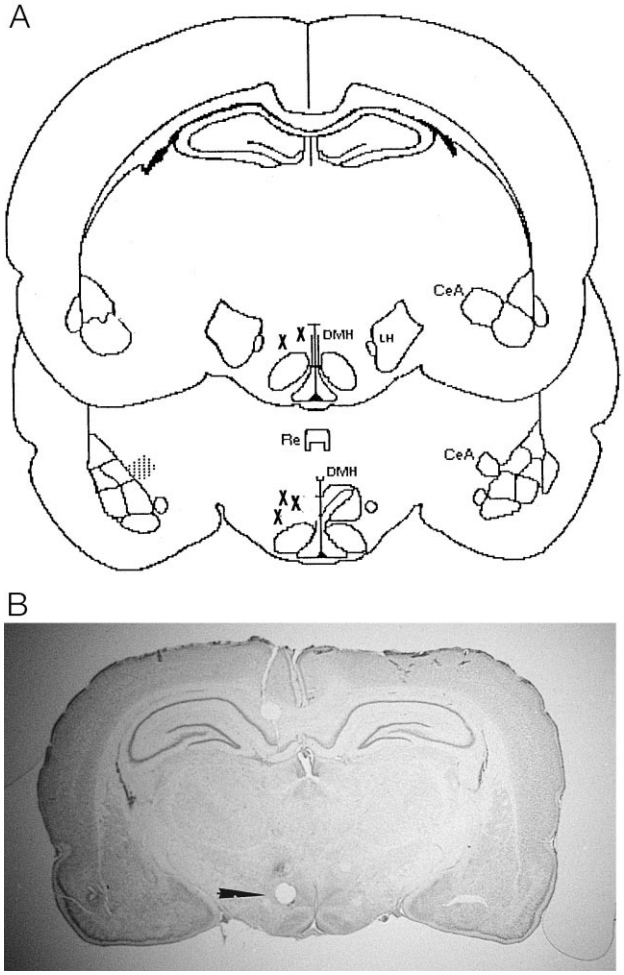

Fig. 1. (A) Schematic coronal serial sections of the region of the dorsomedial hypothalamus (DMH; −2.60 and −3.30 mm posterior to bregma) adapted from Paxinos and Watson (1986). X, microinjection sites; LH, lateral hypothalamus; CeA, central nucleus of amygdala; Re, reuniens thalamic nucleus. (B) Photomicrograph of dorsomedial hypothalamic nucleus showing the microinjection site (arrow).

stained with thionin for light microscopic examination. Only proper cannula placements were included in the study (Fig. 1).

2.4. Drugs

All drugs were dissolved and diluted in 0.9% saline. Bicuculline methiodide, hemicholinium-3, physostigmine salicylate, carbachol, mecamylamine hydrochloride, atropine sulfate, ketamine and urethane were obtained from Sigma (St. Louis, MO, USA). Chlorpromazine was kindly provided by Eczacibaşi (Turkey).

2.5. Data analysis

All results are expressed as means ± S.E.M. Mean arterial pressure (mmHg) was calculated using the formula: Mean arterial pressure = (pulse pressure/3) + diastolic pressure. Data were statistically evaluated by using one-way analysis of variance (ANOVA) for repeated measures. Significant treatment effects were subsequently delineated by using Duncan's post-hoc test. Two-way ANOVA was used for the comparison of time-response curves obtained for different groups. The level of statistical significance was considered to be $P < 0.05$.

3. Results

3.1. Effects of hemicholinium-3 on pressor responses induced by physostigmine and carbachol

The acetylcholine depleting effect of hemicholinium-3 was demonstrated by the injection of intravenous (i.v.) physostigmine (100 μg/kg; $n = 6$) or i.c.v. carbachol (250 ng; $n = 5$). Both physostigmine and carbachol produced a significant increase in mean arterial pressure after saline pre-treatment (Table 1). I.c.v. injection of hemicholinium-3 (9 nmol) almost completely blocked physostigmine-induced blood-pressure elevations, whereas it did not alter those of carbachol (Fig. 2). Since physostigmine-induced blood-pressure changes are mediated via brain acetylcholine, but carbachol acts directly on the muscarinic receptors, these results indicate that there was brain acetylcholine depletion.

Table 1
Time course of mean arterial pressure (MAP; mmHg) due to i.v. administration of physostigmine (Physo; 100 μg/kg) or i.c.v. injection of carbachol (CCh; 250 ng) in animals pre-treated with saline (Sal.) or hemicholinium-3 (HC-3; 5 μg)

Treatment	n	Time (min)								
		−60	0	1	3	5	10	15	20	30
Sal. + Physo.	6	99.3 ± 2.2	99.0 ± 2.3	104.3 ± 3.6	109.6 ± 4.3	112.7 ± 4.7 [a]	108.7 ± 3.1	104.7 ± 2.5	102.5 ± 2.8	99.4 ± 2.1
HC-3 + Physo.	6	97.0 ± 3.6	98.0 ± 2.7	99.3 ± 2.7	100.3 ± 2.5	101.4 ± 3.1	99.1 ± 2.8	98.7 ± 2.6	97.8 ± 2.6	98.7 ± 2.5
Sal. + CCh	5	103.1 ± 1.9	103.0 ± 2.3	117.2 ± 2.2	122.3 ± 2.7 [a]	128.1 ± 1.95 [a]	125.1 ± 2.4 [a]	118.2 ± 2.6 [a]	113.6 ± 2.2 [a]	114.4 ± 4.3
HC-3 + CCh	5	100.3 ± 2.6	101.3 ± 3.1	115.1 ± 3.0	124.0 ± 3.9 [a]	126.2 ± 4.9 [a]	125.5 ± 4.7 [a]	120.9 ± 3.7 [a]	117.0 ± 3.4 [a]	119.9 ± 3.5

Pre-treatment with saline or hemicholinium-3 was administered at 60 min before injection of physostigmine or carbachol. Values are expressed as means ± S.E.M. Asterisks indicate significance of differences from baseline levels (min 0) using one-way ANOVA followed by the post-hoc test of Duncan, [a] $P < 0.05$; n, the number of the animals in each group.

Table 2
Effects of i.c.v. saline, hemicholinium-3 (9 nmol), mecamylamine (245 nmol) or atropine sulfate (15 nmol) pre-treatment on mean arterial pressure (mmHg). I.c.v. injections were given immediately after the zero time point

Treatment	*n*	Baseline levels	30 min after injection	60 min after injection
Saline	6	101.5 ± 1.2	99.8 ± 1.4	100.9 ± 1.8
Hemicholinium-3	6	98.5 ± 1.3	96.1 ± 2.4	96.4 ± 1.6
Mecamylamine	5	106.6 ± 3.1	105.2 ± 4.2	-
Atropine	4	104.0 ± 1.4	102.9 ± 1.5	-

Values are expressed as means ± S.E.M. *n*, the number of the animals in each group.

3.2. Blood-pressure responses to i.c.v. administration of bicuculline methiodide in hemicholinium-3- or saline-pre-treated animals

Saline and hemicholinium-3 administration alone did not alter mean arterial pressure throughout the observation period (Table 2). I.c.v. administration of bicuculline methiodide produced dose-dependent elevations in blood pressure in saline-pre-treated rats (Figs. 3–5). Whereas the lowest dose of bicuculline methiodide (0.05 nmol; $n = 6$) did not cause any significant blood-pressure changes, the pressor responses to the doses of 0.3 ($n = 8$) and 0.5 ($n = 6$) nmol became evident within 1 min after the administration of the drug, reached their maximum in 5 min and returned to the control levels within 20 min. Animals receiving bicuculline methiodide exhibited transient behavioral changes, such as exploration, running and sniffing.

Hemicholinium-3 pre-treatment caused significantly higher pressor responses to 0.3 and 0.5 nmol bicuculline methiodide compared to those seen in saline-pre-treated group when analyzed by two-way ANOVA ($P < 0.05$; Figs. 4 and 5). Furthermore, 0.05 nmol bicuculline methiodide also produced a significant pressor response in hemicholinium-3-pre-treated animals, indicating potentiation (Fig. 3). Two-way ANOVA for the 30-min time course revealed a significant interaction effect ($P < 0.01$). In order to determine whether muscarinic or nicotinic receptors mediate the blood-pressure effects of bicuculline methiodide, pre-treatment with atropine or mecamylamine was given i.c.v. 30 min before the injection of bicuculline

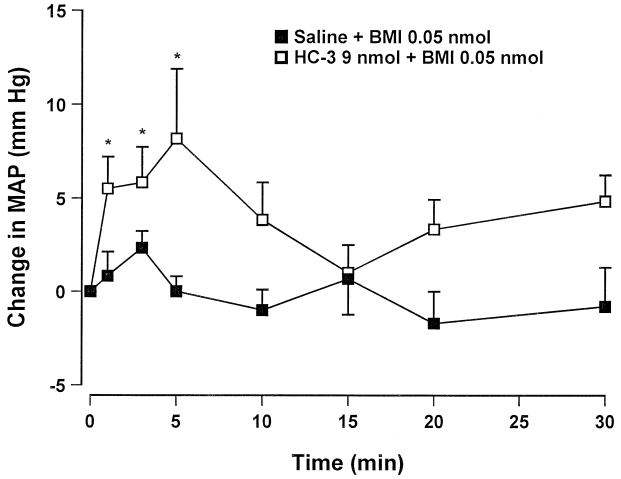

Fig. 3. Time-course of the changes in mean arterial pressure (MAP; mmHg) due to i.c.v. administration of 0.05 nmol bicuculline methiodide (BMI) in animals pre-treated with saline ($n = 6$) or 9 nmol hemicholinium-3 (HC-3; $n = 6$). Values represent means ± S.E.M. Asterisks indicate significance of differences from baseline levels using one-way ANOVA followed by the post-hoc test of Duncan, * $P < 0.05$. Two-way ANOVA indicates a significant overall difference between saline and hemicholinium groups ($P < 0.01$).

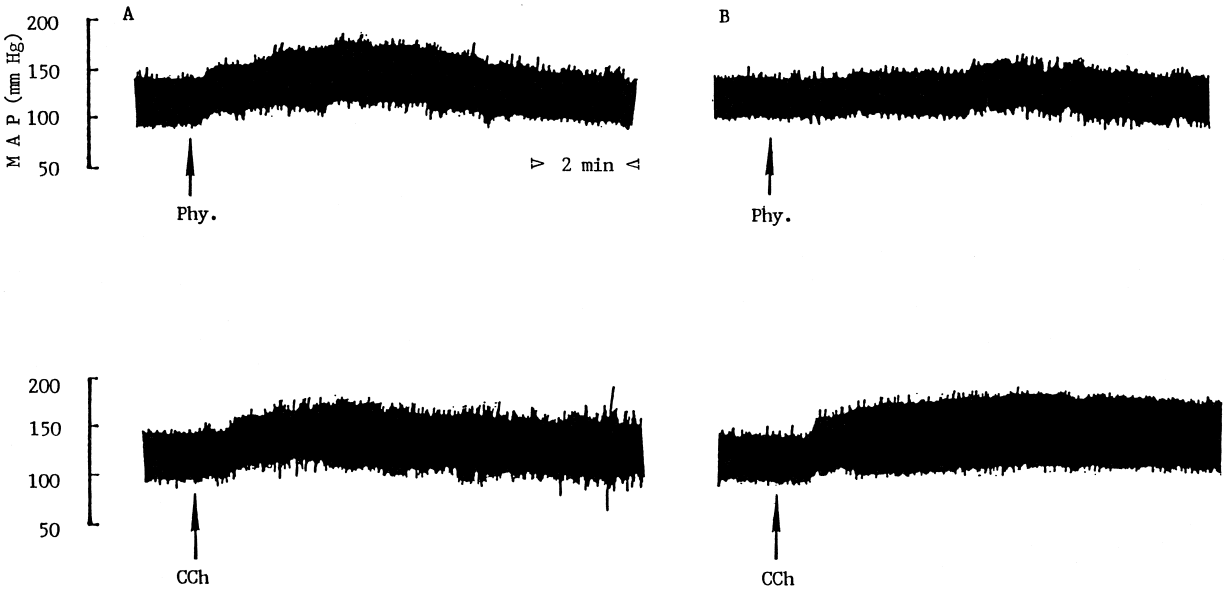

Fig. 2. Representative traces showing mean arterial pressure responses (MAP; mmHg) to i.v. injection of physostigmine (Phy.; 100 μg/kg) or i.c.v. administration of carbachol (CCh; 250 ng) in (A) saline (10 μl i.c.v.)- and (B) hemicholinium-3 (9 nmol i.c.v.)-pre-treated animals. Pre-treatment with saline or hemicholinium was administered at 60 min before the injection of physostigmine or carbachol.

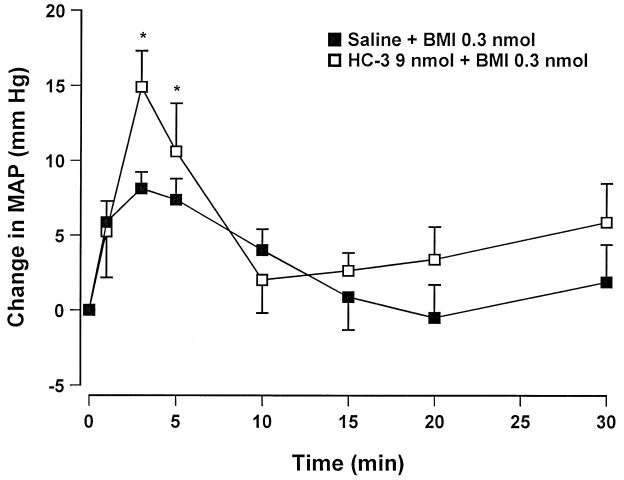

Fig. 4. Time-course of the changes in mean arterial pressure (MAP; mmHg) due to i.c.v. administration of 0.3 nmol bicuculline methiodide (BMI) in animals pre-treated with saline ($n = 8$) or 9 nmol hemicholinium-3 (HC-3; $n = 8$). Values represent means ± S.E.M. Asterisks indicate significance of differences from baseline levels using one-way ANOVA followed by the post-hoc test of Duncan, * $P < 0.05$. Two-way ANOVA indicates a significant overall difference between saline and hemicholinium groups ($P < 0.05$).

methiodide. Neither mecamylamine (245 nmol; $n = 5$) nor atropine (15 nmol; $n = 4$) alone altered the basal levels of blood pressure (Table 2). The dose of 0.05 nmol of bicuculline methiodide caused elevation of blood pressure in rats pre-treated with mecamylamine (Fig. 6). This effect was significantly higher than that seen in the saline group when analyzed by two-way ANOVA ($P < 0.05$). The same dose did not produce any blood-pressure changes in atropine-pre-treated animals (Fig. 6).

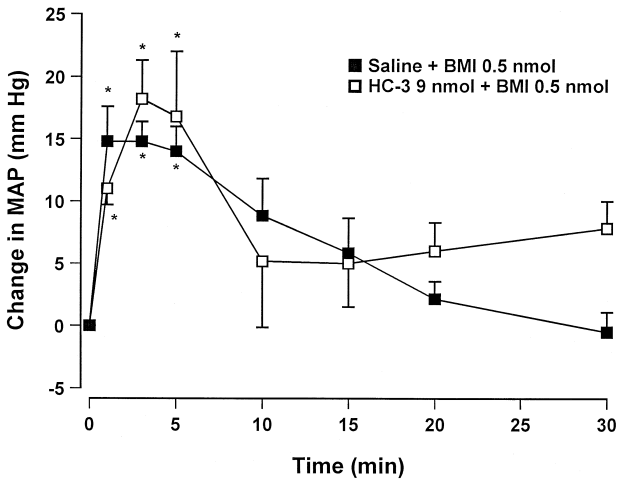

Fig. 5. Time-course of the changes in mean arterial pressure (MAP; mmHg) due to i.c.v. administration of 0.5 nmol bicuculline methiodide (BMI) in animals pre-treated with saline ($n = 6$) or 9 nmol hemicholinium-3 (HC-3; $n = 6$). Values represent means ± S.E.M. Asterisks indicate significance of differences from baseline levels using one-way ANOVA followed by the post-hoc test of Duncan, * $P < 0.05$. Two-way ANOVA indicates a significant overall difference between saline and hemicholinium-3 groups ($P < 0.05$).

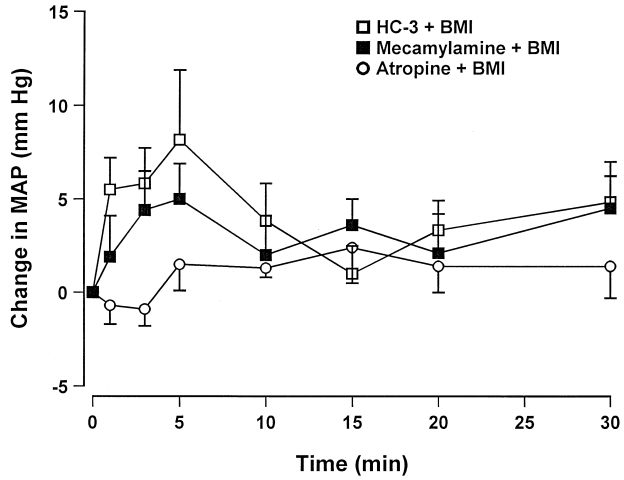

Fig. 6. Time-course of the changes in mean arterial pressure (MAP; mmHg) due to i.c.v. administration of 0.05 nmol bicuculline methiodide (BMI) in animals pre-treated with hemicholinium-3 (HC-3; 9 nmol, $n = 6$), mecamylamine (245 nmol, $n = 5$) or atropine sulfate (15 nmol, $n = 4$). Values represent means ± S.E.M. Two-way ANOVA indicates a significant overall difference between HC-3 + BMI and Atropine + BMI groups ($P < 0.05$).

3.3. Blood-pressure responses to the administration of bicuculline methiodide into the dorsomedial hypothalamic nucleus in hemicholinium-3- or saline-pre-treated animals

Bicuculline methiodide injection into the dorsomedial hypothalamic nucleus at a dose of 40 pmol ($n = 5$) induced a pressor response in control rats that appeared within 30 s, reached its maximum in 3–5 min and lasted for 15–20 min after the injection. The amplitude of the blood-pressure response to bicuculline methiodide was sig-

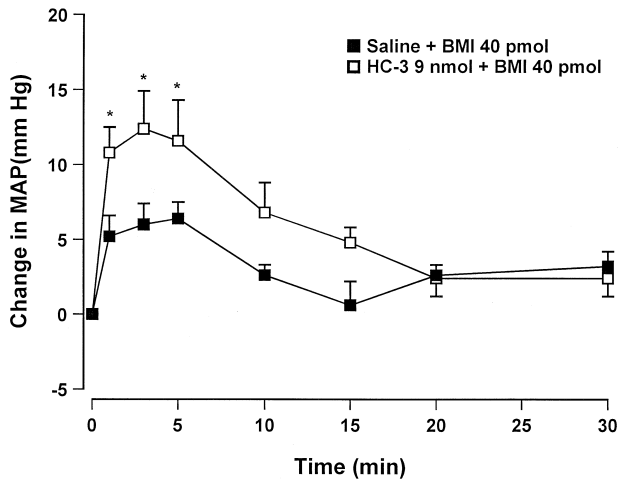

Fig. 7. Time-course of the changes in mean arterial pressure (MAP; mmHg) due to the administration of 40 pmol bicuculline methiodide (BMI) into the dorsomedial hypothalamic nucleus preceded by saline ($n = 5$) or hemicholinium-3 (HC-3; 9 nmol, $n = 5$). Values represent means ± S.E.M. Asterisks indicate significance of differences from baseline levels using one-way ANOVA followed by the post-hoc test of Duncan, * $P < 0.05$. Two-way ANOVA indicates a significant overall difference between saline and hemicholinium groups ($P < 0.001$).

nificantly higher in rats pre-treated with hemicholinium-3 when compared with that observed in saline-pre-treated animals ($P < 0.001$; Fig. 7).

4. Discussion

Central $GABA_A$ receptor blockade by bicuculline methiodide produced a significant and dose-dependent increase in blood pressure. The pressor effect of bicuculline methiodide was also seen when it was injected into the dorsomedial hypothalamic nucleus. The depletion of brain acetylcholine was demonstrated to potentiate blood-pressure elevations induced by both i.c.v. and intrahypothalamic injection of bicuculline methiodide. Cholinergic receptor antagonists, atropine and mecamylamine, were used to characterize the receptor subtype mediating the cholinergic potentiation of the bicuculline methiodide-induced blood-pressure response. The lowest and ineffective dose of bicuculline methiodide was chosen for this purpose. The blockade of nicotinic receptors by mecamylamine, like hemicholinium-3, potentiated the pressor response to bicuculline methiodide, whereas atropine was ineffective.

Gritti et al. (1994) have shown that GABA-synthesizing neurons are co-distributed with acetylcholine-synthesizing neurons within the basal forebrain. A relatively large number of glutamic acid decarboxylase positive cells are distributed throughout the basal forebrain cell groups where choline acetyl transferase positive cells are also located. Leranth and Frotscher (1990) have also demonstrated that glutamic acid decarboxylase-positive terminals form synaptic contacts on choline acetyl transferase-immunoreactive dendrites in the medial septum/diagonal band complex, and cholinergic terminals establish synaptic contacts with glutamic acid decarboxylase immunoreactive cell bodies and proximal dendrites in the medial septum/diagonal band complex as well as in the lateral septum. In this respect, the electrophysiological findings strongly imply the presence of functional muscarinic receptors on GABAergic interneurons and an extensive neural connectivity of the hippocampal muscarinic cholinoceptive non-pyramidal system and the inhibitory GABAergic circuitry (Van Der Zee and Luiten, 1993). Furthermore, they have found that the immunocytochemical staining intensity for muscarinic acetylcholine receptors appears to be considerably higher in GABAergic than in cholinergic neurons, suggesting a stronger cholinergic impact upon the GABAergic neurons (Van Der Zee and Luiten, 1994). Pitler and Alger (1992) have demonstrated that the cholinergic agonist carbachol causes excitation of hippocampal GABAergic non-pyramidal cells. Similarly, the stimulation of cholinergic axons increases GABAergic activity in the CA_1 pyramidal cells (Alger, 1991). It has also been reported that microiontophoretic application of the selective muscarinic agonist, betanechol, increases GABA-induced inhibition of Purkinje cell firing (Andre et al., 1994). An increase of the spontaneous release of [^{3}H]GABA outflow in the rabbit caudate nucleus slices exposed in superfusion to acetylcholine was also observed in vitro by Limberger et al. (1986). The effect of acetylcholine was blocked by hexamethonium but not by atropine, suggesting the involvement of nicotinic receptors.

It was reported that the excitatory amino-acid receptor agonists *N*-methyl-D-aspartic acid (NMDA) and kainic acid produced an increase in heart rate and blood pressure similar to those produced by the $GABA_A$ receptor antagonist bicuculline methiodide when microinjected into the hypothalamus (Soltis and Di Micco, 1991). It has also been shown that the pressor and tachycardic effects of bicuculline methiodide are attenuated by an NMDA receptor antagonist, AP5, and by a non-NMDA receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). These authors have suggested that the cardiovascular effects caused by the blockade of the GABAergic inhibitory system in the dorsomedial hypothalamic nucleus are dependent on the activation of local NMDA and non-NMDA excitatory amino-acid receptors. A cholinergic-GABAergic interaction was also demonstrated at the level of the dorsomedial hypothalamic nucleus in the present study. Furthermore, acetylcholine has been shown to inhibit glutamate release from hippocampal synaptosomes (Marchi et al., 1988; Marchi and Raiteri, 1989) and cortical and striatal slices (Godukhin et al., 1984; Benjamin and Quastel, 1977) via muscarinic receptors. All of these findings strengthen the suggestion that acetylcholine has a stimulatory influence on GABAergic inhibitory mechanisms whereas it inhibits glutamergic activity in the brain regions involved in blood-pressure regulation. Since GABA, like acetylcholine, also suppresses excitatory amino-acid receptor-mediated responses, the blockade of $GABA_A$ receptors in these regions may cause a blood-pressure increase at least partially via excitatory amino-acid neurons. Therefore, the depletion of brain acetylcholine is expected to potentiate bicuculline methiodide-induced pressor effects as a consequence of the withdrawal of the cholinergic stimulatory influence on GABA and inhibitory tone on excitatory amino acid-mediated mechanisms throughout the brain. The results of this study also suggest that acetylcholine has a modulator role on $GABA_A$ receptor-mediated blood-pressure responses via nicotinic receptors.

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