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European Journal of Pharmacology 362 (1998) 173–181

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Effect of muscimol on cholinomimetic-induced cardiovascular responses in rats

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Received 30 March 1998; revised 9 October 1998; accepted 16 October 1998

Abstract

Brain acetylcholine and gamma-aminobutyric acid (GABA) are both involved in the regulation of central cardiovascular control. Despite data from anatomical and electrophysiological experiments characterizing the interaction between central GABAergic and cholinergic neurotransmission, the potential significance of this interaction in central cardiovascular regulation remains unknown. The purpose of this study was to determine whether activation of $GABA_A$ receptors by intracerebroventricular or intrahypothalamic administration of muscimol affects the cholinergic agonist-induced cardiovascular responses. All experiments were performed in conscious, Sprague–Dawley rats instrumented with a guide cannula for drug injection and iliac arterial catheters for direct measurement of mean arterial pressure and heart rate. Administration of a cholinergic agonist, carbachol, either intracerebroventricularly or into the dorsomedial hypothalamic nucleus, produced a significant increase in mean arterial pressure, whereas injection of carbachol into the posterior hypothalamic nucleus caused a slight elevation in blood pressure. Pretreatment with muscimol 10 min before administration of carbachol prevented the carbachol-evoked blood pressure changes. On the other hand, carbachol produced variable changes in heart rate, depending on the site of injection. In [^{3}H]quinuclydinyl benzilate binding experiments, muscimol did not displace the muscarinic radioligand from its binding sites, suggesting that it does not exert any direct antagonistic activity at muscarinic receptors. These results suggest that the dorsomedial hypothalamic nucleus is a potential site of action for microinjected carbachol and that the GABAergic system has an inhibitory influence on cholinergic neurons involved in blood pressure regulation.

Keywords: GABA; (γ-aminobutyric); Cholinergic; Blood pressure; Heart rate; Dorsomedial hypothalamic nucleus; Posterior hypothalamic nucleus

1. Introduction

Immunohistochemical findings strongly suggest the coexistence of gamma-aminobutyric acid (GABA) and acetylcholine in mammalian nerve endings (Van Der Zee and Luiten, 1994). More specifically, Zaborszky et al. (1986) have shown that glutamic acid decarboxylase-immunopositive terminals make synaptic contacts with cholinergic neurons of the rostral forebrain. In addition, several investigators have reported on the codistribution of GABA- and acetylcholine-synthesizing neurons in the basal forebrain of rats, an extensive neuronal connectivity of hippocampal muscarinic cholinergic and the GABAergic systems and the striatal GABAergic medium spiny neurons in contact with large aspiny cholinergic neurons, indicating an important role for GABAergic systems in modulation of the output of the large aspiny cholinergic neurons (Gritti et al., 1993, 1994; Van Der Zee and Luiten, 1993).

In order to further elucidate the relation between GABAergic and cholinergic systems, De Boer and Westerink (1994) studied the effects of GABAergic drugs on the output of striatal acetylcholine, using in vivo brain microdialysis techniques. The results of this study have clearly shown that the GABAergic system tonically inhibits the output of striatal acetylcholine via $GABA_A$ receptors, but not via $GABA_B$ receptors. Likewise, it has been reported that the alteration of plasma epinephrine and norepinephrine concentrations induced by neostigmine was suppressed by intracerebroventricular coadministration of muscimol, suggesting that activation of $GABA_A$ receptors in the central nervous system suppresses sympathetic neural activity and the adrenal medullary response induced by activation of cholinergic neurons (Nonogaki et al., 1994).

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0014-2999/98/$ - see front matter
PII: S0014-2999(98)00754-7

Taken together, the results of these morphological and biochemical studies suggest the importance of bidirectional GABAergic–cholinergic interactions in the central nervous system.

The role of central cholinergic and GABAergic neurons in cardiovascular homeostasis and in the development and maintenance of experimental hypertension has been well demonstrated (Brezenoff and Giuliano, 1982; Brezenoff and Xiao, 1989; Buccafusco, 1996; Gören et al., 1996; Özkutlu et al., 1993; Tellioğlu et al., 1997; Wible et al., 1988). However, little is known about the functional significance of GABA–acetylcholine interactions in the central regulation of cardiovascular functions. Previous studies in our laboratory have provided evidence that endogenous brain acetylcholine has a modulator role in $GABA_A$ receptor-mediated blood pressure control, possibly via nicotinic receptors in the rat (Tellioğlu et al., 1996). Another approach to this issue is to determine the effect of activation of central $GABA_A$ receptors on the cardiovascular responses induced by activation of the central cholinergic neurons. Therefore, the present study was designed to examine the effect of muscimol, a specific $GABA_A$ receptor agonist, on the blood pressure and heart rate changes induced by carbachol, a direct muscarinic cholinergic receptor agonist. The possibility of muscimol acting directly on the muscarinic receptors was also investigated using the displacement of [^{3}H] quinuclydinyl benzilate binding assay.

2. Materials and methods

Female Sprague–Dawley rats weighing 200–250 g were fed a standard laboratory rat chow and tap water ad libitum and housed in Plexiglass cages in a 12-h light/dark cycled and temperature-controlled room (20 ± 3°C). All procedures were approved by the Institutional Animal Care and Use Committee of Marmara University.

2.1. Cannula placement

Three to 4 days before the experiments, the animals were anesthetized with ketamine (50 mg/kg, intraperitoneally; i.p.) and chlorpromazine (1 mg/kg, i.p.). The head of the rat was placed in the stereotaxic apparatus (Stoelting Model 51600, USA). The scalp was longitudinally incised, and the skull was leveled between lambda and bregma. In the first series of experiments, a stainless steel guide cannula (Plastic Ones, System C313G, Roanoke, VA, USA) was placed into the right lateral ventricle (1 mm caudal to bregma, 1.5 mm lateral to the midline and 3.4 mm ventral to the surface of the skull) according to the stereotaxic atlas of Paxinos and Watson (1986). In the next group of experiments, a guide cannula (Plastic Ones, System C315G, Roanoke, VA, USA) was implanted at a 10° angle into the right dorsomedial hypothalamic nucleus (3.1 mm caudal and 2.15 mm lateral to bregma and 8.9 mm ventral to the surface of the skull) or right posterior hypothalamic nucleus (4.2 mm caudal and 2.0 mm lateral to bregma and 8.3 mm ventral to the surface of the skull) using the stereotaxic atlas. The guide cannula was fixed to the skull with dental cement and three screws. A removable stylet plugged the guide cannula except during the time of drug injection.

2.2. Blood pressure and heart rate determination

On the day of the experiment, the rats were lightly anesthetized with ether and 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. An injection stylet extending 1 mm below the tip of the guide cannula was placed for intracerebroventricular or intraparenchymal administration of the vehicle or test compounds. The animal was put in a Plexiglass 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 to record blood pressure on a polygraph (Grass Model 7, USA). Heart rates (beats/min) were obtained via a tachograph (Grass Model 7P44, USA) Following the stabilization period, basal blood pressure and heart rate were measured. Then, drugs were administered either intracerebroventricularly or intraparenchymally in a volume of 10 μl or 150 nl, respectively. Injections were done slowly within 30 s via a Hamilton microsyringe attached to an infusion pump (Kd Scientific, USA) through the extension tubing to avoid handling the animal during the test period.

In the first series of experiments, cardiovascular responses to intracerebroventricular carbachol (0.8 nmol) 10 min after intracerebroventricular administration of saline ($n = 13$) or muscimol (5 nmol; $n = 10$) were determined. In the second series of experiments, carbachol was administered into the dorsomedial hypothalamic nucleus in different doses (0.4 nmol; $n = 5$, 0.8 nmol; $n = 4$ and 1.6 nmol; $n = 9$) after saline pretreatment. In the next group, carbachol (1.6 nmol) was injected into the dorsomedial hypothalamic nucleus 10 min after intrahypothalamic administration of muscimol (150 pmol; $n = 8$ and 450 pmol; $n = 6$) or saline ($n = 9$). Muscimol, alone (150 and 450 pmol; $n = 4$ for each dose) was also injected into the dorsomedial hypothalamic nucleus. In the last group, carbachol (1.6 nmol) was injected into the posterior hypothalamic nucleus 10 min after intrahypothalamic administration of muscimol (150 pmol; $n = 6$) or saline ($n = 6$).

2.3. Histological examination

For the verification of the injection site, the animals were anesthetized with urethane (1.2 mg/kg, i.p.) after the

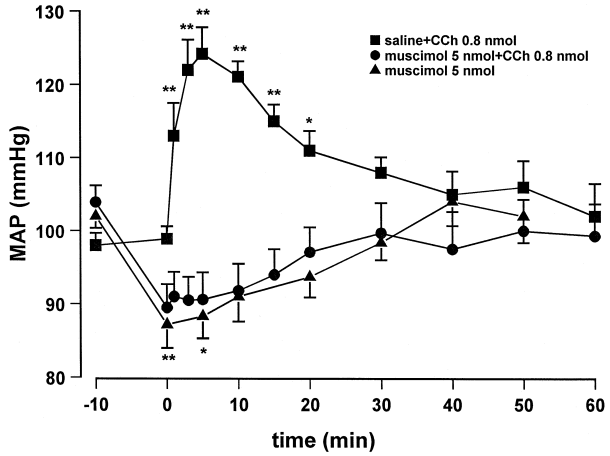

Fig. 1. Time course of mean arterial pressure (MAP; mm Hg) after intracerebroventricular injection of carbachol in saline- or muscimol-pretreated animals. Time course of mean arterial pressure after administration of muscimol alone. Values represent the means ± S.E.M of 10–13 individual experiments. Asterisks indicate significances of differences from baseline levels, using One-way ANOVA followed by post-hoc test of Dunnett. $* p < 0.05$, $** p < 0.01$.

experiment. Methylene blue was injected into the lateral cerebral ventricle, the dorsomedial hypothalamic nucleus or the posterior hypothalamic nucleus in a volume of 10 μl or 150 nl, respectively. The brains were then removed and kept in a 20% sucrose–formalin solution for 1 week. Forty-μm coronal sections were cut through the dorsomedial hypothalamic nucleus region with a cryostat (Microm, FRG) and stained with thionin for light microscopic examination. Only proper cannulae placements were included in the study.

2.4. Drugs

All drugs were dissolved and diluted in 0.9% saline. Carbachol (carbamylcholine chloride), muscimol, ketamine and urethane were purchased from Sigma, St. Louis, MO, USA. Chlorpromazine was kindly provided by Eczacibaşi, Turkey.

2.5. Data analysis

All results were expressed as means ± S.E.M. Mean arterial pressure (mm Hg) was calculated using the formula: mean arterial pressure = 1/3 pulse pressure + diastolic pressure. Heart rate (beats/min) was obtained from direct counting of pulsatile pressure waveforms. The data were statistically evaluated by one-way analysis of variance (ANOVA) for repeated measures. Significant treatment effects were subsequently delineated using the post-hoc Dunnett's test. Two-way ANOVA was used for the comparison of time–response curves obtained from different groups. The level of statistical significance was considered to be $P < 0.05$.

2.6. Radioligand binding assay

The hypothalamic and cortical regions of the brains were removed and homogenized in sodium-potassium phosphate buffer (SPPB, 50 mM) with an Ultra Turrax homogenizer at maximal speed. The homogenate was centrifuged at $1000 \times g$ at 4°C. A portion of membrane suspension containing about 0.1 mg tissue for hypothalamus and 0.2 mg for cortex in 2 ml of SPPB was incubated at 37°C for 90 min with 0.3 nM [^{3}H]quinuclydinyl benzilate (spec. act. 47 Ci/mmol) and increasing concentrations (10^{-10}–10^{-4} M) of atropine ($n = 4$) or muscimol ($n = 4$) in a total volume of 2 ml. The reaction was stopped by filtration through glass fiber filters (Sigma, F-6019), washed with 3 ml of ice-cold SPPB twice, air-dried, kept in 3 ml scintillation fluid overnight and counted in a liquid scintillation analyzer (Packard Tri-Carb 1500) with an efficiency of 58–60%. The filters were pretreated with 0.1% polyethylenimine to reduce the amount of radioactivity retained by the filter. Specific binding is defined as the difference between the radioactivity bound without and with atropine (10^{-6} M).

3. Results

3.1. Cardiovascular responses to intracerebroventricular administration of carbachol in saline- or muscimol-pretreated rats

Intracerebroventricular administration of 5 nmol of muscimol, alone, caused a significant reduction in mean

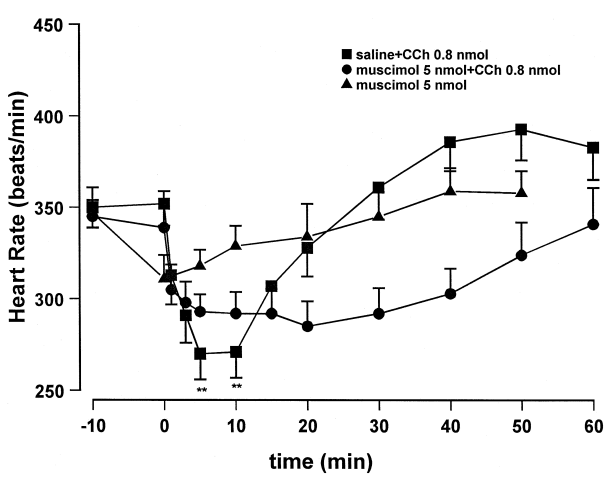

Fig. 2. Time course of heart rate (beats/min) after intracerebroventricular injection of carbachol in saline- or muscimol-pretreated animals. Time course of heart rate after administration of muscimol alone. Values represent the means ± S.E.M of 10–13 individual experiments. Asterisks indicate significances of differences from baseline levels, using One-way ANOVA followed by post hoc test of Dunnett. $* p < 0.05$, $** p < 0.01$.

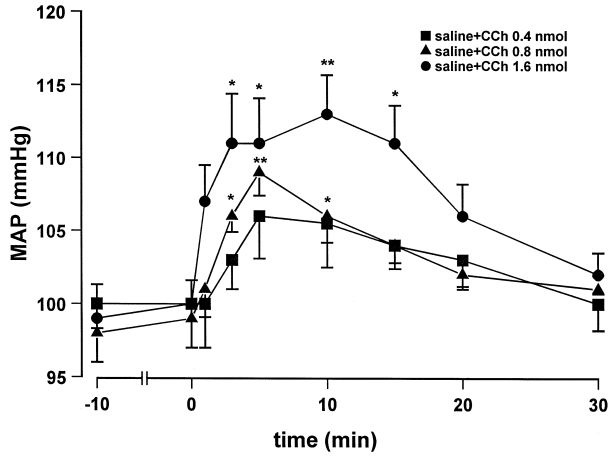

Fig. 3. Time course of mean arterial pressure (MAP; mm Hg) after injection of carbachol into the dorsomedial hypothalamic nucleus in saline-pretreated animals. Values represent the means ± S.E.M of 4–9 individual experiments. Asterisks indicate significances of differences from baseline levels, using One-way ANOVA followed by post hoc test of Dunnett. $* p < 0.05$, $** p < 0.01$.

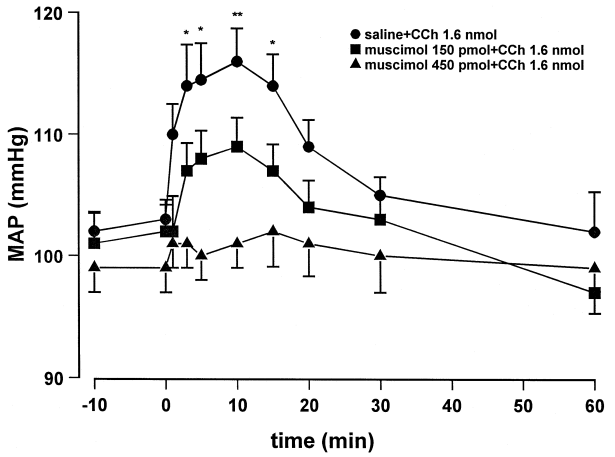

Fig. 5. Time course of mean arterial pressure (MAP; mm Hg) after injection of carbachol into the dorsomedial hypothalamic nucleus in saline- or muscimol-pretreated animals. Values represent the means ± S.E.M of 6–9 individual experiments. Asterisks indicate significances of differences from baseline levels using One-way ANOVA followed by post-hoc test of Dunnett. $* p < 0.05$, $** p < 0.01$.

arterial pressure and a slight but non-significant decrease in heart rate (Figs. 1 and 2). These cardiovascular responses reached a maximum at 10 min. Mean arterial pressure and heart rate then returned to their baseline values by 30 to 60 min post-injection. Intracerebroventricular injection of carbachol in saline-pretreated rats produced statistically significant elevations of mean arterial pressure, reaching peak magnitude by 5 min (Fig. 1). In contrast, significant reductions in heart rate after the injection of carbachol were observed within 30 min (Fig. 2). Interestingly, carbachol given at 10 min after muscimol administration produced no changes in blood pressure. The significant pressor response to carbachol was completely abolished by prior injection of muscimol (Fig. 1). Therefore, two-way ANOVA for the 60-min time course revealed a significant interaction effect ($P < 0.05$). On the other hand, muscimol pretreatment did not significantly alter the maximal heart rate response to carbachol but caused a sustained bradycardia for nearly 30 min. Carbachol was given at 10 min after muscimol injection since the maximal cardiovascular responses to muscimol alone occurred at 10 min after administration. No overt locomotor changes were observed after intracerebroventricular administration of carbachol or muscimol. The rats either slept or rested quietly during the experimental period.

3.2. Cardiovascular responses to carbachol injected into the hypothalamus in saline- or muscimol-pretreated rats

Administration of carbachol (0.4, 0.8 and 1.6 nmol) into the dorsomedial hypothalamic nucleus 10 min after the

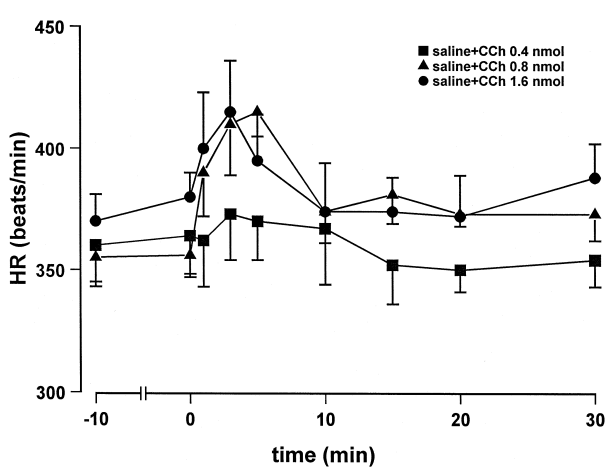

Fig. 4. Time course of heart rate (beats/min) after injection of carbachol into the dorsomedial hypothalamic nucleus in saline-pretreated animals. Values represent the means ± S.E.M. of 4–9 individual experiments.

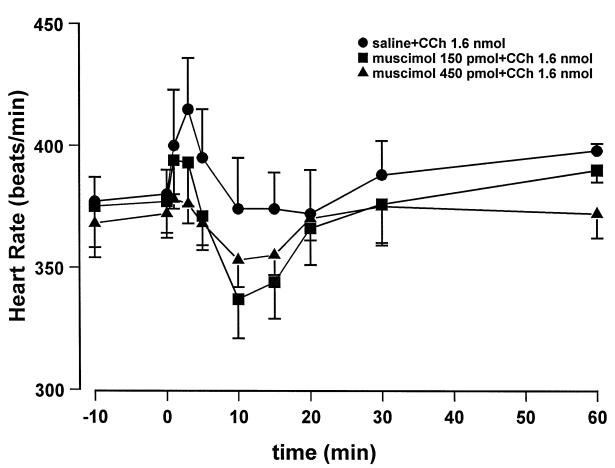

Fig. 6. Time course of heart rate (beats/min) after injection of carbachol into the dorsomedial hypothalamic nucleus in saline- or muscimol-pretreated animals. Values represent the means ± S.E.M of 6–9 individual experiments.

Table 1
Time course of mean arterial pressure (MAP; mm Hg) and heart rate (HR; beats/min) after the injection of muscinol, alone, into the DMH

Treatment		*n*	Time (min)						
Muscimol			0	1	3	5	10	15	30
150 pmol	MAP	4	103 ± 2.4	101 ± 2.8	101 ± 3.0	102 ± 3.1	102 ± 3.4	102 ± 2.1	100 ± 3.1
	HR		368 ± 10.0	361 ± 10.7	358 ± 17.3	368 ± 12.4	386 ± 18.5	379 ± 19.2	368 ± 19.5
450 pmol	MAP	4	97 ± 1.0	96 ± 1.0	96 ± 1.4	95 ± 1.0	94 ± 1.0	95 ± 1.6	99 ± 1.4
	HR		338 ± 8.1	330 ± 7.1	326 ± 11.9	326 ± 10.4	323 ± 6.7	315 ± 10.6	346 ± 11.5

Values are expressed as mean ± S.E.M.
n indicates the number of the animals in each group.

saline injection caused dose-dependent elevations of blood pressure and heart rate (Figs. 3 and 4). The significant pressor response to carbachol became evident within 1 min, reached its maximum in 10 min and returned to the control levels within 30 min.

Muscimol (150 and 450 pmol) was microinjected into the dorsomedial hypothalamic nucleus 10 min prior to the administration of 1.6 nmol of carbachol also in the dorsomedial hypothalamic nucleus (Figs. 5 and 6). The pressor response to carbachol in muscimol-pretreated animals (150 pmol) was smaller than that in the saline-pretreated group. Likewise, a higher dose of muscimol (450 pmol) injected into the dorsomedial hypothalamic nucleus totally prevented the carbachol-induced pressor response. Two-way ANOVA indicated a significant overall difference between saline- and muscimol-pretreated groups ($P < 0.05$). The non-significant tachycardiac response to the dose of 1.6 nmol of carbachol was also prevented with the dose of 450 pmol of muscimol (Fig. 6). Muscimol, alone, at the same doses had no effect on mean arterial pressure and heart rate (Table 1).

Intraparenchymal injection of 1.6 nmol of carbachol into the region of the posterior hypothalamic nucleus produced a slight but non-significant elevation of blood pressure and heart rate (Figs. 7 and 8). Administration of muscimol into the posterior hypothalamic nucleus also prevented the slight pressor response to carbachol. The pressor response to carbachol microinjected into the posterior hypothalamic nucleus was smaller than that observed after administration into the dorsomedial hypothalamic nucleus.

No overt locomotor or behavioral changes were observed after the intraparenchymal injection of muscimol whereas microinjection of carbachol into the nuclei of hypothalamus caused an increase in locomotor activity.

3.3. Binding experiments

Fig. 9 shows the displacement of [^{3}H] quinuclydinyl benzilate specific binding by muscimol or atropine, a non-specific muscarinic receptor antagonist, in cerebrocortical and hypothalamic homogenates. [^{3}H] Quinuclydinyl benzilate binding was displaced by increasing concentrations of atropine (10^{-10}–10^{-4} M). However, muscimol in

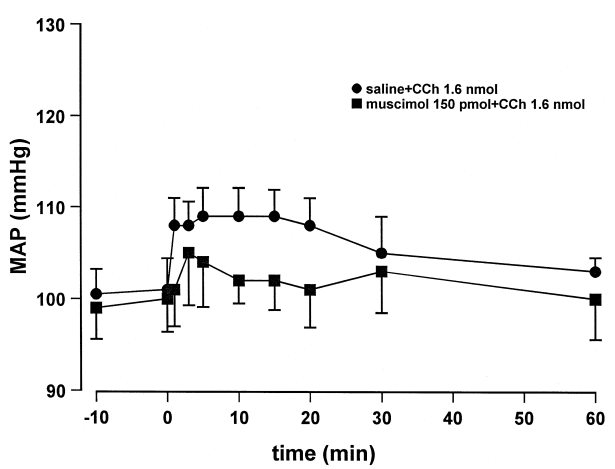

Fig. 7. Time course of mean arterial pressure (MAP; mm Hg) after injection of carbachol into the posterior hypothalamic nucleus in saline- or muscimol-pretreated animals. Values represent the means ± S.E.M of 6 individual experiments.

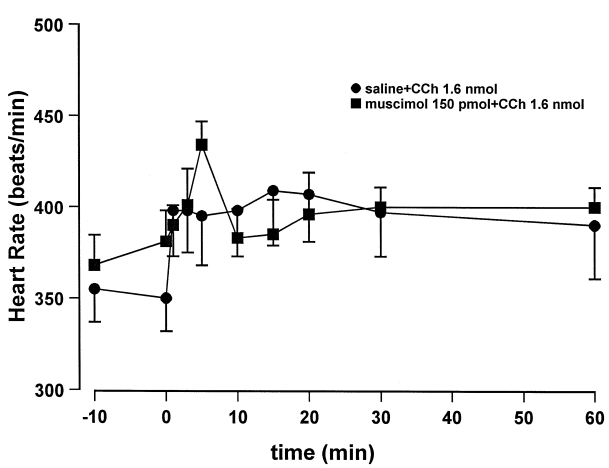

Fig. 8. Time course of heart rate (beats/min) after injection of carbachol into the posterior hypothalamic nucleus in saline- or muscimol-pretreated animals. Values represent the means ± S.E.M of 6 individual experiments.

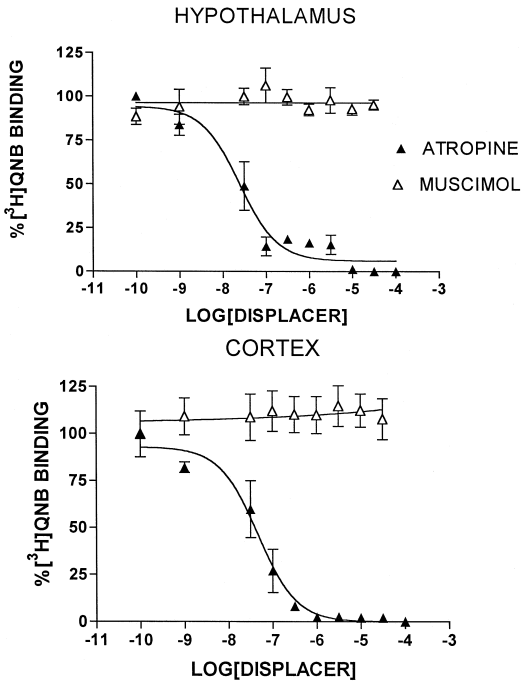

Fig. 9. Displacement of [^{3}H]-QNB specific binding by muscimol (10^{-10}–10^{-4} M; $n=4$) or atropine (10^{-10}–10^{-4} M; $n=4$).

increasing concentrations (10^{-10}–10^{-4} M) failed to displace [^{3}H] quinuclydinyl benzilate from its binding sites.

4. Discussion

The outcomes of this study showed that microinjection of muscimol either intracerebroventricularly or into the dorsomedial hypothalamic nucleus prevented carbachol-evoked blood pressure changes in conscious rats (see Fig. 10). It seems possible that cholinergic activity in the regulation of blood pressure could be inhibited by activation of $GABA_A$ receptors. This is in agreement with previous reports that administration of $GABA_A$ receptor agonists into the forebrain structures, or given systemically, results in a decrease of acetylcholine release, acetylcholine turnover and high-affinity choline uptake (De Boer and Westerink, 1994; Zsilla et al., 1976; Wood and Richard, 1982). Furthermore, the effect of GABA on the release of acetylcholine was studied in vitro by Stoof et al. (1979). It has been reported that GABA reduced the depolarization-induced release of acetylcholine from slices of rat brain. In this respect, Supavilia and Karobath (1985) have also demonstrated that $GABA_A$ receptor agonists, muscimol and 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3(2H)one (THIP), inhibit acetylcholine release from striatal slices, suggesting that there is a modulation of acetylcholine release by GABA in vitro. The results of our study also appear to support previous hypotheses about the functional significance of the GABA-cholinergic link in the central nervous system (Dudchenko and Sarter, 1991; Zarrindast et al., 1995).

On the other hand, radioligand binding experiments were performed to investigate whether muscimol, as a $GABA_A$ receptor agonist, acts directly on central muscarinic receptors. Quinuclydinyl benzilate is a very well known and very widely used muscarinic ligand preferred for radioligand binding experiments. In this respect, muscimol failed to displace the muscarinic radioligand from its binding sites, whereas [^{3}H] quinuclydinyl benzilate binding was displaced by atropine, a non-specific muscarinic antagonist, suggesting that this $GABA_A$ receptor agonist does not exert any direct antagonistic activity at muscarinic receptors in hypothalamus and cortex homogenates.

The central cholinergic system is well known to be involved in cardiovascular regulation (Brezenoff and Giuliano, 1982). Activation of brain cholinergic neurotransmission results in an increase in blood pressure in several species, including rat and man (Aslan et al., 1997; Brezenoff and Xiao, 1989; Ulus et al., 1995). Sites responsive to microinjection of cholinomimetics in terms of blood pressure elevation include the posterior hypothalamic nucleus, ventrolateral medullary pressor area, anteroventral third ventricle (AV3V) region, locus coeruleus and lateral septum (Buccafusco, 1996; Menani et al., 1990; Onat et al., 1994; Sundaram et al., 1988). The hypothalamus, as a forebrain site, is one of the areas regulating central cardiovascular function and containing high levels of acetylcholine (Cambell and Jenden, 1970; Uchimura et al., 1975). Results of previous studies have suggested that acetylcholine in the posterior hypothalamic nucleus serves to mediate a rise in arterial blood pressure (Brezenoff and Xiao, 1989; Buccafusco and Brezenoff, 1979; Martin, 1992). It has been shown that the injection of 5 nmol of carbachol into the posterior hypothalamic nucleus increases blood pressure, while the same dose of carbachol outside the posterior hypothalamic nucleus (regions lying approximately 0.5 mm lateral or caudal from the border of the nucleus) fails to evoke a change in blood pressure, indicating that the carbachol-induced pressor response is mediated from the posterior hypothalamic nucleus (Martin, 1992). The posterior nucleus in the hypothalamus had, however, first been suggested as the site of action for microinjected cholinomimetics. Our findings in the present study also implicate the dorsomedial hypothalamic nucleus in carbachol-induced blood pressure changes, since administration of carbachol into the dorsomedial hypothalamic nucleus caused an increase in mean arterial pressure greater than that seen after injection into the posterior hypothalamic nucleus.

On the other hand, administration of 0.8 nmol of carbachol into the dorsomedial hypothalamic nucleus evokes a lower blood pressure rise than that seen with the intracerebroventricular route. When carbachol is injected into the cerebral ventricles, it diffuses to more than one brain structure involved in cardiovascular regulation. In other

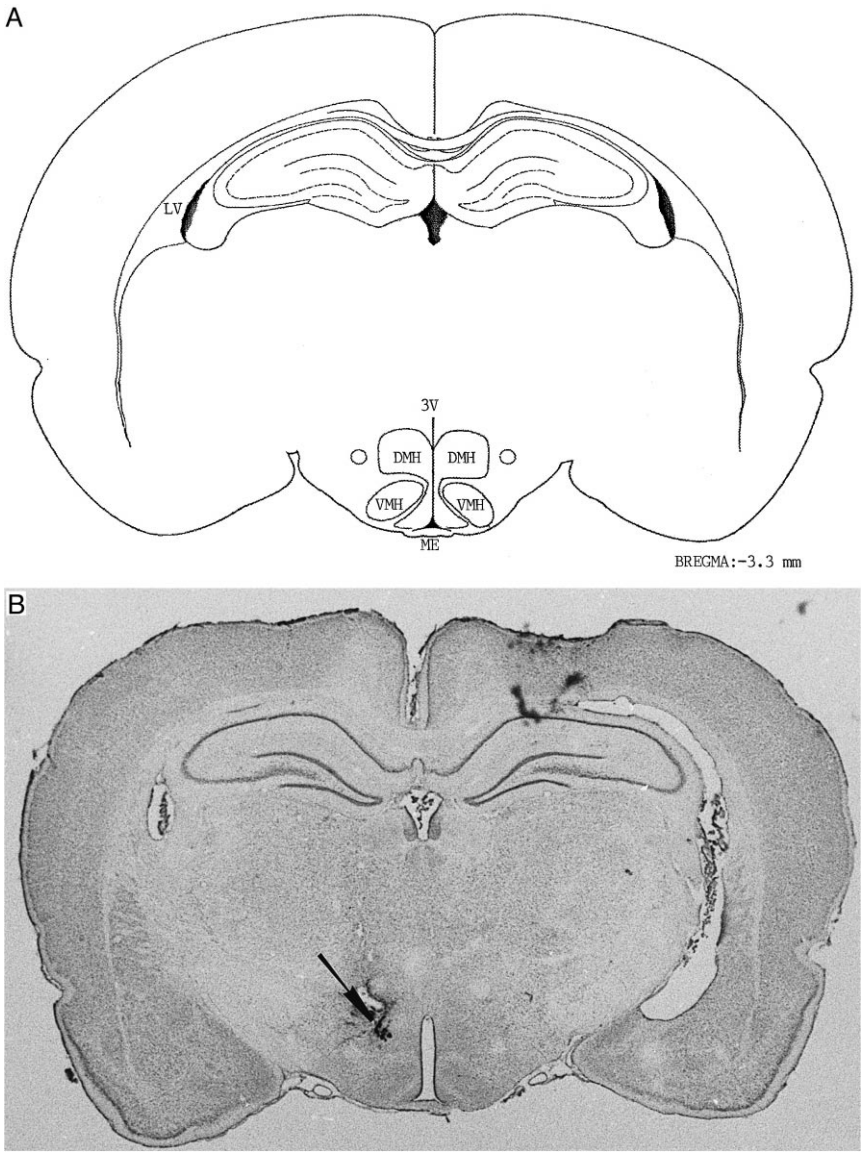

Fig. 10. (A) Schematic coronal section of the region of the dorsomedial hypothalamic nucleus (DMH; −3.3 mm posterior to bregma) adapted from the rat brain atlas of Paxinos and Watson. LV, Lateral ventricle; ME, Median Eminence; VMH, Ventromedial hypothalamic nucleus. (B) Photomicrograph of the dorsomedial hypothalamic nucleus. Arrow shows the microinjection site.

words, intracerebroventricular administration of this agent results in a gross cardiovascular effect originating from multiple sites of the brain. Microinjection of carbachol directly into specific sites of the brain, such as the dorsomedial nucleus of the hypothalamus produces an effect originating from that particular region. As shown in Figs. 1 and 3, the kinetics of the effect of 0.8 nmol of carbachol given either into the dorsomedial hypothalamic nucleus or intracerebroventricularly are similar, reaching a maximum magnitude at 5 min after the injection. However, the blood pressure effect of a higher dose of carbachol (1.6 nmol) injected into the dorsomedial hypothalamic nucleus is much longer lasted than that observed with 0.4 or 0.8 nmol of carbachol. The longer duration of the blood pressure response to 1.6 nmol at 10 and 15 min suggests the possibility of a diffusion to the third ventricle, although carbachol was injected in a very small volume, 150 nl.

Interestingly, changes in heart rate were found to be variable, depending on the site of administration. Injection of carbachol into the dorsomedial hypothalamic nucleus produced a slight increase in heart rate, whereas administration into the lateral cerebral ventricle caused a statistically significant fall in the value of this parameter. A possible explanation for this observation is that the bradycardiac response to intracerebroventricular administration of carbachol is the sum of its effects at different sites in the brain and is not mediated through the hypothalamus. Furthermore, Taira and Enero (1989) have reported that

centrally administered cholinergic drugs, neostigmine and bethanechol, induce bradycardia in conscious rats, whereas bradycardiac responses to cholinergic agents are not observed in sinoaortic-denervated rats, suggesting that the bradycardia induced by the cholinergic stimulation may be mediated by a baroreflex mechanism. In addition, these authors had also shown previously that baroreceptor deafferentation affects the peripheral parasympathetic tone to the heart and produces changes in cholinergic pathways involved in the heart rate response. On the other hand, the bradycardia seen after intracerebroventricular injection of carbachol was not significantly but slightly prevented by muscimol pretreatment. One possibility is that $GABA_A$ receptors do not have an essential role in cholinergic heart rate regulation.

In conclusion, our results clearly indicate that cholinergic activation of the dorsomedial hypothalamic nucleus mediates a significant rise in arterial blood pressure, thus suggesting that the dorsomedial hypothalamic nucleus is among the cholinergic centers involved in central cardiovascular regulation. In addition, activation of $GABA_A$ receptors by muscimol administration completely prevents the pressor effects of carbachol, possibly by depressing cholinergic activity in the brain.

Acknowledgements

This work was supported by the Scientific and Technical Research Council of Turkey (TÜBİTAK, project no. SBAG-AYD-13).

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