ELSEVIER

European Journal of Pharmacology 336 (1997) 163–168

ejp

Role of the AV3V region in the pressor responses induced by amygdala stimulation

Tahir Tellioğlu, Neslihan Aslan, Zafer Gören, Filiz Onat, Şule Oktay *

Department of Pharmacology, Marmara University School of Medicine, Haydarpasa, 81326 Istanbul, Turkey

Received 1 July 1997; revised 29 July 1997; accepted 1 August 1997

Abstract

The role of the anteroventral third ventricle (AV3V) region in the pressor responses to carbachol injected into the lateral cerebral ventricle (i.c.v.), the electrical stimulation of and carbachol-induced stimulation of, the central nucleus of the amygdala were investigated in conscious, unrestrained Sprague–Dawley rats. I.c.v. and intra-amygdalar carbachol caused a significant rise in blood pressure of 22.9 ± 2.8 and 16.8 ± 2.2 mmHg, respectively. Electrical stimulation (1 ms, 80 Hz, 50–300 μA, for 30 s) of the central nucleus of amygdala also produced intensity-dependent pressor effects. Electrolytic lesion of the AV3V region abolished the pressor responses induced by carbachol and by electrical amygdala stimulation. The heart rate changes were also significantly inhibited in the AV3V-lesioned rats. These results indicate that the integrity of the AV3V region is essential for the central cholinergic cardiovascular changes induced by central amygdaloid nucleus stimulation. © 1997 Elsevier Science B.V.

Keywords: Amygdala; AV3V region; Cholinergic hypertension; Carbachol; Blood pressure; Heart rate

1. Introduction

The stimulation of central muscarinic receptors in rats results in hypertension, primarily through an increase in sympathetic outflow to the vasculature (Brezenoff and Giuliano, 1982). The administration of cholinomimetics directly into the cerebral ventricles or into several specific sites such as the posterior hypothalamic nucleus, the ventrolateral medullary pressor area, hippocampus, locus ceruleus and C1 area of the rostral ventrolateral medulla was reported to induce a pressor response (Buccafusco and Brezenoff, 1979; De Luca et al., 1990; Giuliano et al., 1989; Haruta et al., 1992; Martin, 1992; Nattie and Li, 1990; Sundaram et al., 1988).

The amygdaloid complex was shown to have rich neuronal connections with structures in the hypothalamus and the medulla oblongata and has been implicated in the control of several autonomic functions including cardiovascular control (Dampney, 1994). The injection of carbachol into the amygdaloid complex was reported to elicit a pressor response (Aslan et al., 1997a; Ohta et al., 1991). The presence of cholinergic neurons and muscarinic receptors in the amygdala was demonstrated by measuring choline acetyltransferase activity and acetylcholine concentrations and by an autoradiographic muscarinic receptor binding assay (Hoover et al., 1978; Rotter et al., 1979). It was speculated that certain aspects of cardiovascular control integrated through the amygdala may involve the cholinergic system through by muscarinic receptors (Aslan et al., 1997a; Ohta et al., 1991).

The anteroventral third ventricle (AV3V) region is known to be important in the central control of cardiovascular homeostasis and body fluid balance in rats. It was shown that AV3V lesions prevent salt-induced hypertension in borderline hypertensive rats (Sanders and Johnson, 1989), but reduce adrenergic vascular compensation during hemorrhagic shock (Schaumloffel et al., 1990). This region was also demonstrated to be essential for pressor, dipsogenic and natriuretic responses to the central administration of carbachol (Menani et al., 1990).

This study was designed to investigate the role of the AV3V region in the pressor responses to carbachol-induced stimulation and electrical stimulation of the central nucleus of the amygdala in conscious rats. The concomitant changes in heart rate were also investigated.

* Corresponding author. Tel. and Fax: (90-216) 347-5594; e-mail: suleok@escortnet.com

2. Materials and methods

2.1. Animals

Experiments were performed on albino Sprague–Dawley rats of both sexes weighing 200–250 g. All animals were fed a standard diet with water, ad libitum, and kept at room temperature (20 ± 3°C) in an air-conditioned room with a 12 h light/dark cycle. Since AV3V lesions may cause adipsia and diminished food intake lasting for several weeks, the animals were observed closely and given extra care after AV3V lesioning. The body weights were measured before AV3V lesioning and on the day of the experiment. Weight loss was not more than 10% and no significant difference between the sham-lesioned and AV3V lesioned animals was detected.

2.2. Surgical procedures

Rats were anesthetized with ketamine (50 mg/kg, i.p.) and chlorpromazine (1.0 mg/kg, i.p.) and the head was fixed in a stereotaxic apparatus (Stoelting Model 51600). Cannula and/or electrode placements and lesion generation were performed as follows:

(a) AV3V lesion: An electrolytic AV3V lesion was made according to the technique described by Buggy and Johnson (1977). Briefly, a monopolar insulated stainless steel wire electrode (0.4 mm in diameter) bare at the tip was implanted into the AV3V region (0.0–0.3 mm posterior to the bregma, in the midline and 8.5 mm ventral to the surface of the skull) on the basis of the stereotaxic atlas of Paxinos and Watson (1982). An anodal lesion was made with a 3 mA current for 20 s using a DC lesion generator (Grass DC-LM5). A clip attached to the ear was used as the indifferent electrode. The sham-lesioned rats had the electrode placed along the same coordinates except that the depth was 7 mm from the surface of the skull and no electrical current was passed.

(b) Intracerebroventricular cannula placement: After the removal of the AV3V electrode, a guide cannula made from a stainless steel tube (Plastic Products System 313) was implanted into the left lateral cerebroventricle (1.0 mm caudal and 1.5 mm lateral to bregma, 3.2 mm ventral to the surface of the skull; Paxinos and Watson, 1982).

(c) Electrode and cannula placement to the central nucleus of amygdala: A guide cannula-electrode (0.25 mm in diameter) bare at the tip or a 26-gauge stainless steel guide cannula was implanted into the right central nucleus of the amygdala (2.3 mm caudal to bregma, 4.2 mm lateral to the midline and 8.0 mm ventral to the surface of the skull; Paxinos and Watson, 1982) after the removal of the AV3V electrode. An injection stylet extending 1 mm below the tip of the guide cannula was inserted for parenchymal administration of the drug.

The guide cannulas and electrodes were fixed with dental cement together with three screws driven into the skull and were plugged with a removable stylet except during drug injections. The experiments were performed 3–4 days after the AV3V lesioning in order to avoid the influence of local edema that may occur during the electrode placement and lesioning procedures.

2.3. Experimental protocol

Three to five days after lesion generation and cannula or electrode placement, the animals were anesthetized with ether and a polyethylene catheter (PE 10 attached to PE 50), filled with heparin/saline (500 U/ml) solution, was inserted into the abdominal aorta through the right femoral artery for direct blood pressure recordings. The other end of the tubing was passed beneath the skin and exteriorized through an incision in the nape of the neck. A stainless steel wire plug was placed in the exposed ends of the catheters until the experiment. Each animal was moved into a Plexiglass cage ($25 \times 25 \times 30$ cm). Experiments were conducted in conscious and freely moving rats at least 2 h after surgery.

The arterial cannula was connected to a pressure transducer (Grass Model P23ID) and arterial blood pressure was recorded with a polygraph (Grass Model 7). Heart rates (bpm) were obtained via a tachograph (Grass Model 7P44). Following the 2 h stabilization period, the basal arterial blood pressure and heart rate values were established. Then carbachol was injected i.c.v. or into the central nucleus of the amygdala, or electrical amygdala stimulation was performed in intact, sham-operated or AV3V-lesioned rats. I.c.v. injections were done slowly within 20 s in a final volume of 10 μl via a 25 μl microsyringe (Hamilton). Intra-amygdalar injections were done slowly within 1 min in a final volume of 200 nl via a 1 μl microsyringe (Hamilton) through the cannula connected to an infusion pump (Kds Scientific, USA). The central nucleus of the amygdala was stimulated by using a stimulator (Grass, Model S88, USA) through the cannula-electrode implanted 3–5 days before the experiment. Electrical stimulation consisted of 1 ms pulses at 80 Hz for 30 s, at an intensity of 50–300 mA. The guide cannula served as an anode.

At the end of the experiment, the animals were transcardially perfused with 4% buffered formalin solution. The brains were then removed, 50 mm coronal sections were cut through the amygdala region by using a cryostat (Microm, FRG) and stained with thionin for light microscopic examination. The proper position of the electrodes and lesions were confirmed (Figs. 1 and 2). Methylene blue was injected i.c.v. (10 μl) or into the central nucleus of amygdala (200 nl) after the experiment for the verification of cannula placement. Only proper AV3V lesions,

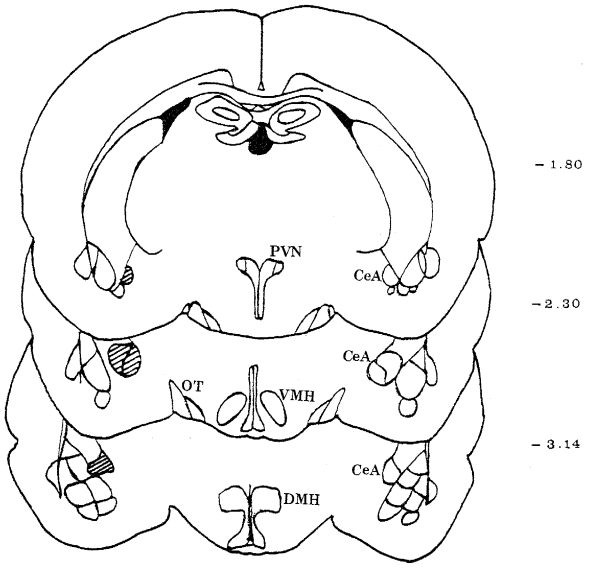

Fig. 1. The sites of stimulation (dashed area) shown on diagrams of a coronal section through the ventral forebrain (1.80, 2.30 and 3.14 mm posterior to bregma). CeA, central nucleus of amygdala; DMH, dorsomedial hypothalamic nucleus; OT, optic tract; PVN, paraventricular nucleus; VMH, ventromedial hypothalamic nucleus.

electrode and cannula placements were included in the study. All procedures were approved by the Institutional Animal Care and Use Commitee.

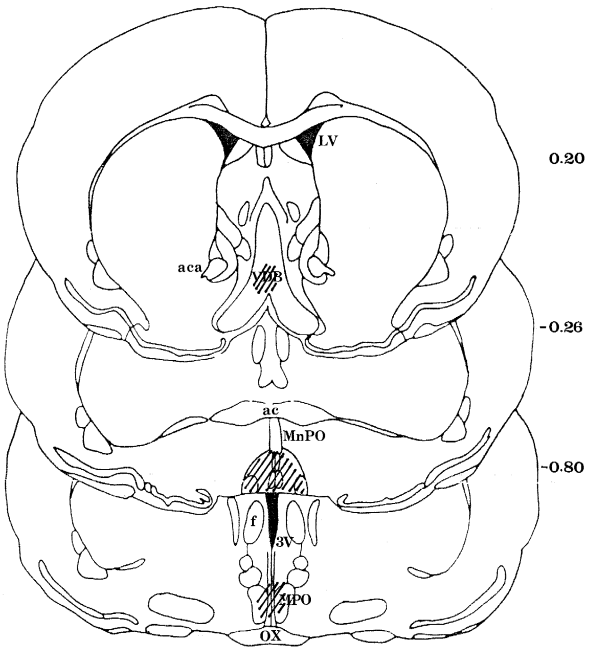

Fig. 2. The lesion sites (dashed area) shown on diagrams of a coronal section through the ventral forebrain (0.2 mm anterior, 0.40 and 0.80 mm posterior to bregma). LV, lateral ventricle; 3V, 3. ventricle; ac, anterior commissure; aca, part of anterior commissure, anterior; f, fornix; MnPO, median preoptic nucleus; MPO, medial preoptic nucleus; OX, optic chiasm.

2.4. Drugs

The following drugs were used: Ketamine HCl (Sigma, USA), chlorpromazine HCl (a gift from Eczacıbaşı, Turkey), carbachol (Sigma, USA), heparin sodium (Liquemine, a gift from Roche, Turkey). All drugs were dissolved and diluted in saline.

2.5. Data analysis

The results were expressed as 'means ± S.E.M.' for 4–7 rats in each group. Mean arterial pressure was calculated as '1/3 pulse pressure + diastolic blood pressure'. The areas under the pressor, tachycardia and bradycardia response–time curves were calculated according to the trapezoidal rule. Data was analyzed by a one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test. The criterion for statistical significance was $P < 0.05$.

3. Results

The basal mean arterial pressure and heart rate values were 97.2 ± 1.0 mmHg and 356.8 ± 5.2 bpm in all animals ($n = 49$). These basal values were not significantly different from each other between groups.

3.1. Effects of carbachol on blood pressure and heart rate

I.c.v. injection of carbachol (250 ng) evoked an increase of 22.9 ± 2.8 mmHg in the blood pressure and a decrease of 41.7 ± 10.5 bpm in the heart rate in intact rats (Figs. 3 and 4). Both pressor and bradycardia responses appeared immediately, reached their maximum 5–10 min after carbachol injection and disappeared within 30 min.

Carbachol, when injected into the central nucleus of the amygdala at a dose of 5 nmol, caused a 16.8 ± 2.2 mmHg

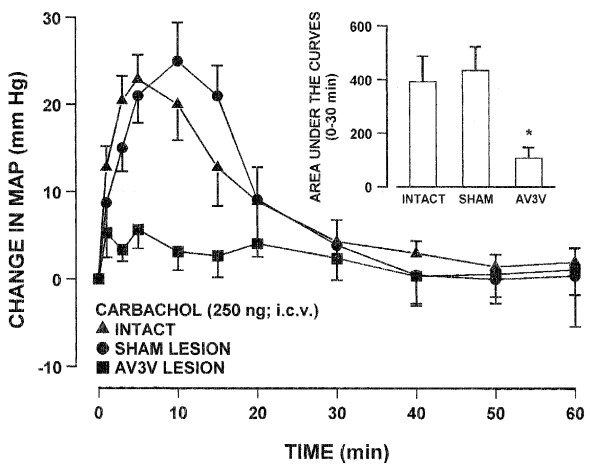

Fig. 3. Influence of i.c.v. injection of carbachol (250 ng) on mean arterial pressure (MAP) in intact ($n = 6$), sham-lesioned ($n = 5$) and AV3V-lesioned ($n = 6$) conscious rats. * $P < 0.05$ versus sham-lesioned group.

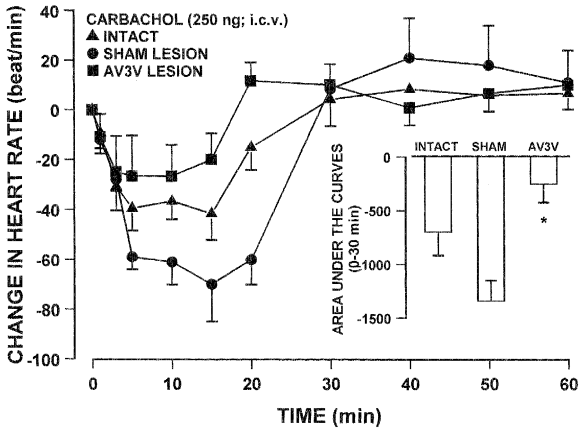

Fig. 4. Influence of the i.c.v. injection of carbachol (250 ng) on heart rate in intact ($n = 6$), sham-lesioned ($n = 5$) and AV3V-lesioned ($n = 6$) conscious rats. * $P < 0.05$ versus sham-lesioned group.

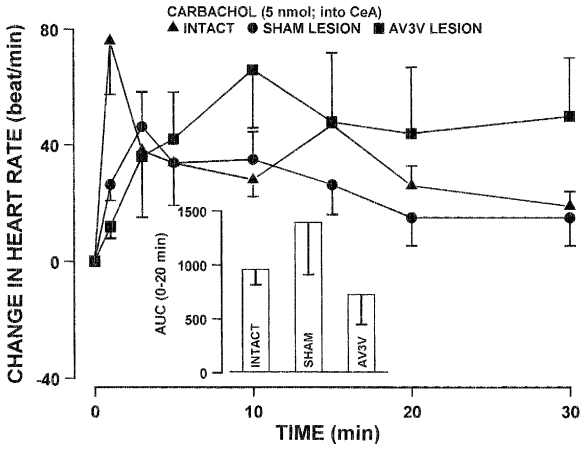

Fig. 6. Influence of carbachol (5 nmol) injected into the central nucleus of the amygdala (CeA) on heart rate (HR) in intact ($n = 5$), sham-lesioned ($n = 4$) and AV3V-lesioned ($n = 5$) conscious rats. AUC represents the areas under the curves. * $P < 0.05$ versus intact rats.

increase in the mean arterial pressure (Fig. 5). The pressor response started immediately, reached its maximum at 3–5 min and the arterial pressure returned to its basal values within 30 min. Although carbachol tended to produce tachycardia in these animals, the overall increase in heart rate did not reach significance (Fig. 6).

Sham-lesioned rats had an increase in mean arterial pressure (21.2 ± 5.0 mmHg) and a decrease in heart rate (65.3 ± 13.2 bpm) in response to i.c.v. injection of 250 ng of carbachol, not significantly different from those observed in intact rats (Figs. 3 and 4). Similarly, the pressor response to carbachol injection into the central nucleus of the amygdala (12.8 ± 1.5 mmHg) was not significantly different from that in the control rats in the sham-lesioned group (Fig. 5).

Carbachol did not cause any significant change in blood pressure and heart rate in rats with an AV3V lesion (Figs. 3–6).

3.2. Effects of electrical amygdala stimulation on blood pressure and heart rate

Electrical stimulation of the central nucleus of the amygdala produced pressor effects in both intact and sham-lesioned rats (Fig. 7). A rapid rise in blood pressure was seen immediately after the initiation of electrical stimulation and the blood pressure returned to its basal values as soon as the stimulation was stopped. The amplitude of the pressor response increased with the intensity of the electrical stimulus (Fig. 7) and was 13.7 ± 2.9 and 19.3 ± 1.9 mmHg with 300 μA in intact and sham-lesioned rats, respectively. The intact and sham-lesioned animals showed a tendency for tachycardia in response to the electrical stimulation of the central nucleus of the amygdala, but did not reach statistical significance in the

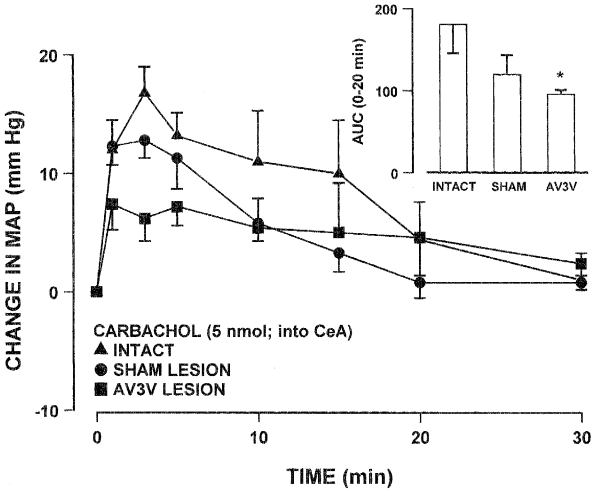

Fig. 5. Influence of carbachol (5 nmol) injected into the central nucleus of the amygdala (CeA) on mean arterial pressure (MAP) in intact ($n = 5$), sham-lesioned ($n = 4$) and AV3V-lesioned ($n = 5$) conscious rats. AUC represents the areas under the curves. * $P < 0.05$ vs intact rats.

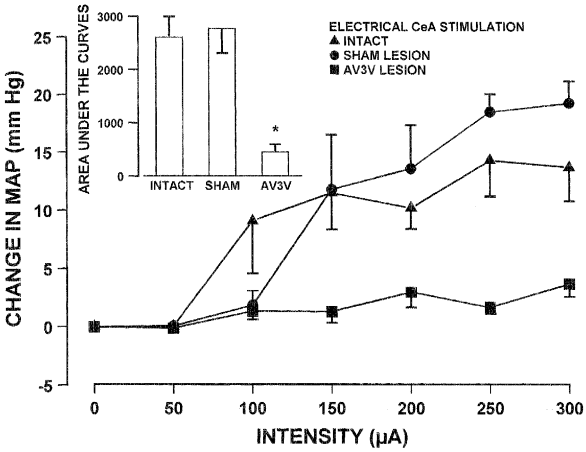

Fig. 7. Influence of electrical stimulation of the central nucleus of amygdala (CeA) on mean arterial pressure (MAP) in intact ($n = 5$), sham-lesioned ($n = 5$) and AV3V-lesioned ($n = 7$) conscious rats. * $P <$ 0.05 versus sham-lesioned group.

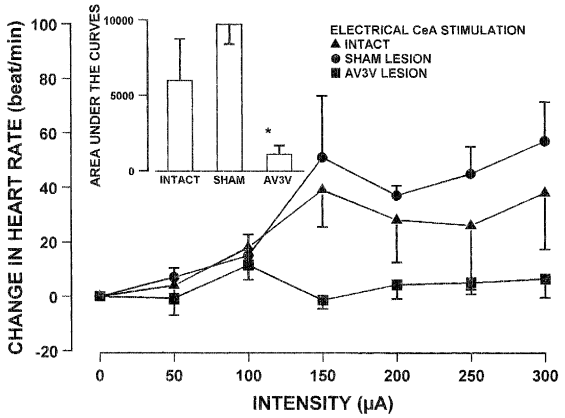

Fig. 8. Influence of electrical stimulation of the central nucleus of the amygdala (CeA) on heart rate in intact (n = 5), sham-lesioned (n = 5) and AV3V-lesioned (n = 7) conscious rats. * $P < 0.05$ vs sham-lesioned group.

intact group because of great inter-individual variation (Fig. 8). The AV3V lesion totally abolished the blood pressure and heart rate changes induced by amygdala stimulation (Figs. 7 and 8).

4. Discussion

Cholinergic agonists evoke qualitatively different cardiovascular responses from different regions of the brain. Brain and brainstem centers known to be involved in cholinergic cardiovascular regulation are the posterior hypothalamus (Brezenoff and Jenden, 1969; Brezenoff and Wirecki, 1970; Buccafusco and Brezenoff, 1979; Martin, 1992), the ventrolateral medullary pressor and depressor areas (Giuliano et al., 1989; Murugaian et al., 1989; Nattie and Li, 1990; Sundaram et al., 1988), the nucleus tractus solitarii (Criscione et al., 1983; Sundaram et al., 1989), the AV3V region (Menani et al., 1990), the locus ceruleus (De Luca et al., 1990), the amygdala (Aslan et al., 1997a; Ohta et al., 1991) and the hippocampus (Haruta et al., 1992). Stimulation of muscarinic receptors of the ventrolateral medullary depressor area and the nucleus tractus solitarii causes hypotension and bradycardia whereas most of the other regions mediate cholinomimetic-induced pressor responses in rats.

The amgydala has been implicated in the control of several autonomic functions including cardiovascular control. Ablation of the amygdala was reported to delay the development of hypertension and attenuate the exaggerated pressor responses to noise stress in rats (Galeno et al., 1982, 1984). Both depressor and pressor effects have been produced by the electrical stimulation of different parts of the amygdaloid complex (Gelsema et al., 1987). It was reported that stimulation in the area of the central nuclei of the amygdala produced a significant increase in blood pressure in conscious rats (Aslan et al., 1997b; Gelsema et al., 1987). In the present study, electrical stimulation of the central nucleus of the amygdala was demonstrated to cause pressor responses that increased in magnitude with the intensity of the electrical current. Although we have demonstrated a tendency to tachycardia, consistent heart rate changes have not been reported either by these authors (Gelsema et al., 1987) or from the present study.

On the other hand, chemical stimulation of the amygdala with carbachol, but not with serotonin and noradrenaline, was found to elicit a pressor response in unanesthetized, unrestrained rats (Ohta et al., 1991). We also reported previously and have now demonstrated that carbachol injected into the central nucleus of the amygdala produces pressor responses (Aslan et al., 1997a). Changes in heart rate were found to be variable. Since these effects were antagonized by atropine (Ohta et al., 1991), pirenzepine and AF-DX 116 (Aslan et al., 1997a), it was concluded that the cholinergic system, via muscarinic receptors in the amygdala, may play a role in the control of cardiovascular function. The presence of acetylcholine, choline acetyltransferase and acetylcholinesterase activities and of muscarinic receptors in the amygdaloid complex was demonstrated by immunohistochemical and radioligand receptor binding studies (Hoover et al., 1978; Rotter et al., 1979). Furthermore, in two recent studies, we have shown that electrolytic ablation of the central nucleus of the amygdaloid complex and the injection of pirenzepine into the central nucleus of the amygdala significantly attenuated carbachol-induced pressor responses (Aslan et al., 1997b; Özkutlu et al., 1995).

The present report demonstrates that ablation of the AV3V region abolished the cardiovascular changes in response to both carbachol-induced and electrical stimulation of the central nucleus of the amygdala in conscious, unrestrainted rats. Several previous studies have also shown the involvement of the AV3V region in central cholinergic cardiovascular control. The AV3V lesions were reported to inhibit pressor, dipsogenic and natriuretic responses to i.c.v. administration of carbachol (Menani et al., 1990) as demonstrated here for the pressor effect. The cardiovascular effects of carbachol injected into the ventromedial hypothalamus and the subfornical organ of rats were significantly reduced by the electrolytic ablation of this region (Collombari et al., 1992; Valladao et al., 1992). AV3V ablation was also reported to attenuate sympathetic nervous system activation due to the nucleus tractus solitarii lesions (Catelli and Sved, 1988) and to reduce adrenergic vascular compensation during hemorrhagic shock (Schaumloffel et al., 1990). Finally, the oxotremorine-induced pressor responses and reversal of hemorrhagic shock were partially inhibited by AV3V lesions indicating that this region plays an important role in cholinergic cardiovascular control in hypotensive animals as well as normotensives (Onat et al., 1994).

In summary and conclusion, we now report that the cardiovascular effects of carbachol injected into the central

nucleus of the amygdala and of electrical stimulation of this area were prevented by AV3V lesions. These results indicate that the integrity of the AV3V region is essential for the central cholinergic cardiovascular changes mediated via the central nucleus of the amygdala.

Acknowledgements

This work was supported by a grant from the Scientific and Technical Research Council of Turkey (project # SBAG-1250).

References

Aslan, N., Gören, Z., Özkutlu, U., Onat, F., Oktay, Ş., 1997a. Modification of the pressor response elicited by carbachol and electrical stimulation of the amygdala by muscarinic antagonists in conscious rats. Br. J. Pharmacol. 121, 35–40.

Aslan, N., Gören, Z., Onat, F., Oktay, Ş., 1997b. Carbachol-induced pressor responses and muscarinic M1 receptors in the central nucleus of amygdala in conscious rats. Eur. J. Pharmacol. 333, 63–67.

Brezenoff, H.E., Giuliano, R., 1982. Cardiovascular control by cholinergic mechanisms in the central nervous system. Annu. Rev. Pharmacol. Toxicol. 22, 341–381.

Brezenoff, H.E., Jenden, D.J., 1969. Modification of arterial blood pressure in rats following microinjection of drugs into the posterior hypothalamus. Int. J. Neuropharmacol. 8, 593–600.

Brezenoff, H.E., Wirecki, T.S., 1970. The pharmacological specificity of muscarinic receptors in the posterior hypothalamus of the rat. Life Sci. 9, 99–109.

Buccafusco, J.J., Brezenoff, H.E., 1979. Pharmacological study of a cholinergic mechanism within the rat posterior hypothalamic nucleus which mediates a hypertensive response. Brain Res. 165, 295–310.

Buggy, J., Johnson, A.K., 1977. Preoptic hypothalamic periventricular lesions: Thirst deficits and hypernatremia. Am. J. Physiol. 233, R44–R52.

Catelli, J.M., Sved, A.F., 1988. Lesions of the AV3V region attenuate sympathetic activation but not the hypertension elicited by destruction of the nucleus tractus solitarius. Brain Res. 439, 330–336.

Collombari, D.S.A., Saad, W.A., Camargo, L.A.A., Renzi, A., De Luca, L.A., Colombari, E., Menani, J.V., 1992. AV3V lesion impairs responses induced by cholinergic activation of SFO in rats. Am. J. Physiol. 263, 1277–1283.

Criscione, L., Reis, D.J., Talman, W.T., 1983. Cholinergic mechanisms in the nucleus tractus solitarii and cardiovascular regulation in the rat. Eur. J. Pharmacol. 88, 47–55.

Dampney, R.A.L., 1994. Functional organization of central pathways regulating the cardiovascular system. Physiol. Rev. 74, 323–364.

De Luca, L.A., Franci, C.R., Saad, W.A., Camargo, L.A.A., Antunes-Rodrigues, J., 1990. Natriuresis induced by cholinergic stimulation of locus coeruleus in the rat. Physiol. Behav. 47, 605–610.

Galeno, T.M., Van Hoesen, G.W., Maixner, W., Johnson, A.K., Brody, M.J., 1982. Contribution of the amygdala to development of spontaneous hypertension. Brain Res. 246, 1–6.

Galeno, T.M., Van Hoesen, G.W., Brody, M.J., 1984. Central amygdaloid nucleus lesion attenuates exaggerated hemodynamic responses to noise stress in the spontaneously hypertensive rat. Brain Res. 291, 249–259.

Gelsema, A.J., McKitrick, D.J., Calaresu, F.R., 1987. Cardiovascular responses to chemical and electrical stimulation of amygdala in rats. Am. J. Physiol. 253, R712–R718.

Giuliano, R., Ruggiero, D.A., Morrison, S., Ernsberger, P., Reiss, D.J., 1989. Cholinergic regulation of arterial pressure by the C1 area of the rostral ventrolateral medulla. J. Neurosci. 9, 923–942.

Haruta, K., Iguchi, A., Matsubara, T., Itoh, K., Che-Lang, C., Yoshida, S., Terada, R., Kanashiro, M., Suzuki, O., Nishimura, H., Sakamoto, N., 1992. Stimulation of muscarinic cholinoceptive neurons in the hippocampus evokes a pressor response with bradycardia. Life Sci. 50, 427–433.

Hoover, D.B., Muth, E.A., Jacobowitz, D.M., 1978. Mapping of the distribution of acetylcholine, choline acetyltransferase and acetylcholinesterase in discrete areas of rat brain. Brain Res. 153, 295–306.

Martin, J.R., 1992. Pressor response to posterior hypothalamic administration of carbachol is mediated by muscarinic M_3 receptor. Eur. J. Pharmacol. 215, 83–91.

Menani, J.V., Saad, W.A., Camargo, L.A.A., Renzi, A., De Luca, L.A., Colombari, E., 1990. The anteroventral third ventricle (AV3V) region is essential for pressor, dipsogenic and natriuretic responses to central carbachol. Neurosci. Lett. 113, 339–344.

Murugaian, J., Sundaram, K., Sapru, H., 1989. Cholinergic mechanism in the ventrolateral medullary depressor area. Brain Res. 501, 355–363.

Nattie, E.E., Li, A., 1990. Ventral medulla sites of muscarinic receptor subtypes involved in cardiorespiratory control. J. Appl. Physiol. 69, 33–41.

Ohta, H., Watanabe, S., Ueki, S., 1991. Cardiovascular changes induced by chemical stimulation of the amygdala in rats. Brain Res. Bull. 26, 575–581.

Onat, F., Aslan, N., Gören, Z., Özkutlu, U., Oktay, Ş., 1994. Reversal of hemorrhagic shock in rats by oxotremorine: The role of muscarinic and nicotinic receptors, and AV3V region. Brain Res. 660, 261–266.

Özkutlu, U., Coşkun, T., Onat, F., Yeğen, B.Ç., Oktay, Ş., 1995. Cardiovascular effects of centrally active cholinomimetics in conscious and anesthetized rats: The role of amygdala. Brain Res. Bull. 37, 569–573.

Paxinos, G., Watson, C., 1982. The rat brain in stereotaxic coordinates. Academic Press, San Diego, CA.

Rotter, A., Birdsall, N.J.M., Burgen, A.S.V., Field, P.M., Hulme, E.C., Raisman, G., 1979. Muscarinic receptors in the central nervous system of the rat. I. Technique for autoradiographic localization of the binding of [^{3}H]propylbenzilycholine mustard and its distribution in the forebrain. Brain Res. 1, 141–165.

Sanders, B.J., Johnson, A.K., 1989. Lesions of the anteroventricular third ventricle prevent salt-induced hypertension in the borderline hypertensive rat. Hypertension 14, 619–622.

Schaumloffel, V., Pugh, V., Bealer, S.L., 1990. Preoptic hypothalamic lesions reduce adrenergic vascular compensation during hemorrhagic shock. Circ. Shock 31, 193–202.

Sundaram, K., Krieger, A.J., Sapru, H., 1988. M_2 muscarinic receptors mediate pressor responses to cholinergic agonists in the ventrolateral medullary pressor area. Brain Res. 449, 141–149.

Sundaram, K., Murugaian, J., Watson, M., Sapru, H., 1989. M_2 muscarinic receptor agonists produce hypotension and bradycardia when injected into the nucleus tractus solitarii. Brain Res. 477, 358–362.

Valladao, A.S., Saad, W.A., Camargo, L.A.A., Renzi, A., De Luca, L.A., Menani, J.V., 1992. AV3V lesion reduces the pressor, dipsogenic, and natriuretic responses to ventromedial hypothalamus activation. Brain Res. Bull. 28, 909–914.