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Carbachol-induced pressor responses and muscarinic M_1 receptors in the central nucleus of amygdala in conscious rats

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Abstract

The type of muscarinic receptor in the central nucleus of the amygdala that mediates the carbachol-evoked pressor responses was investigated in conscious unrestraint Sprague–Dawley rats. Carbachol (100 ng) injected into the lateral cerebral ventricle caused a significant rise in blood pressure of 31.8 ± 4.5 mmHg and a decrease in heart rate of 80.0 ± 12.2 beats/min. Pirenzepine (10–75 nmol) injected into the central nucleus of the amygdala inhibited carbachol-induced pressor responses dose-dependently. The bradycardic response to carbachol was also inhibited by pirenzepine, but no dose-dependency was observed. Injection of pirenzepine into the basolateral amygdala at a dose (50 nmol) that inhibited carbachol-induced changes in mean arterial pressure and heart rate when injected into the central nucleus of the amygdala failed to exert any inhibition. Methoctramine at a dose of 50 nmol injected into both the central nucleus of the amygdala and the basolateral amygdala did not cause any significant alteration in the responses. These results indicate that muscarinic M_1 receptors in the central nucleus of the amygdala are involved in cardiovascular regulation mediated by central cholinergic pathways. © 1997 Elsevier Science B.V.

Keywords: Muscarinic M_1 receptor; Carbachol; Blood pressure; Amygdala, central nucleus of; (Rat)

1. Introduction

Centrally acting acetylcholine receptor agonists and cholinesterase inhibitors are known to induce pressor (Dirnhuber and Cullumbine, 1955; Varagic, 1955; Krstic and Djurkovic, 1973; Ozawa and Uematsu, 1976; Buccafusco and Brezenoff, 1979; Brezenoff and Giuliano, 1982; Oktay et al., 1984; Sundaram et al., 1988; Özkutlu et al., 1993) and depressor responses (De Wildt and Porsius, 1981; Criscione et al., 1983; Murugaian et al., 1989; Sundaram et al., 1989; Hara et al., 1992; Ally et al., 1993) associated with tachycardia or bradycardia depending on the species studied and the mode of administration. In rats, activation of central muscarinic receptors results primarily in hypertension (see Buccafusco, 1996). This occurs when cholinomimetics are administered into the cerebral ventricles or microinjected into the following brain areas, posterior hypothalamic nucleus (Brezenoff and Jenden, 1969; Brezenoff and Wirecki, 1970; Brezenoff, 1972; Buccafusco and Brezenoff, 1979; Martin, 1992), AV3V region (Nattie and Li, 1990; Menani et al., 1990), hippocampus (Haruta et al., 1992), locus coeruleus (De Luca et al., 1990), C1 area of the rostral ventrolateral medulla (Sundaram et al., 1988; Giuliano et al., 1989) and the amygdaloid complex (Ohta et al., 1991; Aslan et al., 1997).

The amygdaloid complex forms dense neuronal connections with the hypothalamus and brainstem, areas that are known to play an important role in cardiovascular regulation (see Dampney, 1994). The general assumption is that the amygdala has a function in the coordination of behavioral and autonomic responses to environmental stimuli. The complex has been demonstrated to contain acetylcholine and acetylcholinesterase (Hoover et al., 1978) and muscarinic binding sites (Rotter et al., 1979). Electrical and chemical stimulation of the central nucleus of the amygdala elicits pressor responses in conscious rats (Gelsema et al., 1987; Ohta et al., 1991). Further, the pressor response evoked by the cholinomimetic carbachol (i.c.v.) is suppressed by electrolytic lesions of the central nucleus of the amygdala (Özkutlu et al., 1995). The present study was designed to investigate the type of muscarinic receptor in the central nucleus of the amygdala that

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mediates the carbachol-evoked pressor response in conscious rats.

2. Materials and methods

2.1. Animals

Experiments were performed in albino Sprague–Dawley rats of both sexes weighing 200–250 g. All animals were fed on a standard diet and water ad libitum. The rats were kept at room temperature (20 ± 3°C) in an air-conditioned room with a 12 h light–dark cycle.

2.2. Surgical procedures

Rats were anesthetized with ketamine (100 mg/kg, i.p.) and chlorpromazine (1.0 mg/kg, i.p.) and the heads were fixed in a stereotaxic apparatus (Stoelting Model 51600). A guide cannula composed of a stainless steel tube (System 313, Plastic One) was implanted into the left lateral cerebral ventricle (1 mm caudal to bregma, 1.5 mm lateral to the midline, and 3.4 mm ventral to the surface of the skull) on the basis of the stereotaxic atlas of Paxinos and Watson (1982). Another guide cannula was placed into the right central nucleus of the amygdala (2.5 mm caudal to bregma, 4.2 mm lateral to the midline and 7.2 mm ventral to the surface of the skull) or basolateral amygdala (2.6 mm caudal to bregma, 4.8 mm lateral to the midline and 7.6 mm ventral to the surface of the skull) (Fig. 1). The injection stylets which extended 1 mm below the tips of the guide cannulas were inserted for drug administrations. The cannulas were fixed by dental cement together with three screws driven into the skull and plugged with a removable stylet except at the time of drug injections.

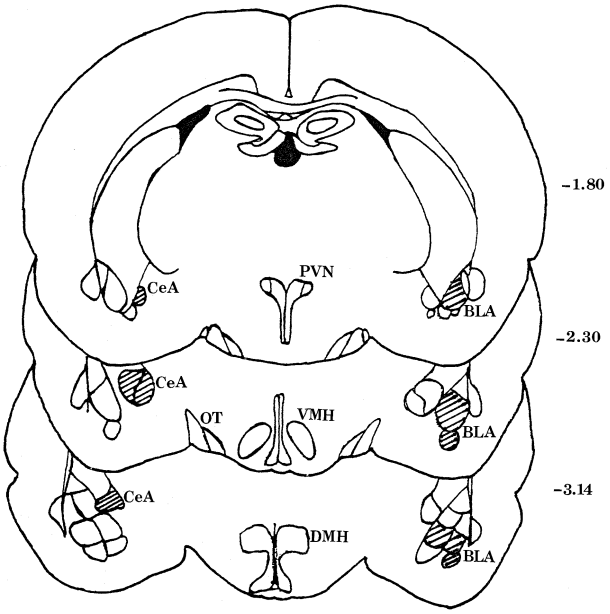

Fig. 1. The sites of antagonist injection (hatched area) shown on diagrams of coronal section through the ventral forebrain (1.80, 2.30 and 3.14 mm posterior to bregma). BLA, basolateral amygdala; CeA, central nucleus of the amygdala; DMH, dorsomedial hypothalamic nucleus; OT, optic tract; PVN, paraventricular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

Three to five days after this surgical intervention, 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. A stainless steel wire plug was placed on the exposed end of the catheter until the experiment. Each animal was moved into a plexiglass cage (25 × 25 × 30 cm). Experiments were conducted at least 2 h after the operation in conscious and freely moving rats.

2.3. Experimental protocols

On the day of the experiment, the arterial cannula was connected to a pressure transducer (Model P23ID, Grass Instruments, Quincy, MA, USA) and arterial blood pressure was recorded on a polygraph (Model 7, Grass Instruments). Heart rate was recorded continuously via a tachograph (Model 7P44, Grass Instruments). Following a 2 h stabilization period, basal arterial blood pressure and heart rate were established. Saline, pirenzepine or methoctramine was injected either into the central nucleus of the amygdala or basolateral amygdala in a volume of 200 nl within 15 s via an infusion pump (Model A.99, Razel, Italy). The experimental groups were designed as follows: (1) Saline ($n = 4$), pirenzepine (10, 25, 50 and 75 nmol; $n = 4$–8 for each dose) or methoctramine (50 nmol; $n = 5$) was injected into the central nucleus of the amygdala. (2) Pirenzepine (50 nmol; $n = 4$) or methoctramine (50 nmol; $n = 4$) was injected into the basolateral amygdala. All rats were injected with i.c.v. carbachol (100 ng) in a volume of 10 μl within 15 s, 10 min after saline or antagonist administration. Arterial blood pressure was monitored for 30 min after carbachol injection.

2.4. Histological examination

At the end of the experiments, 200 nl Indian ink was injected into the central nucleus of the amygdala and basolateral amygdala and 10 μl into the lateral cerebral ventricle, and the animals were transcardially perfused with 4% buffered formaline solution under urethane anesthesia (1.2 g/kg, i.p.). Brains were removed, i.c.v. placements were verified macroscopically and brains were kept in 20% sucrose and formaline solution for 1 week. Then, 40 μm coronal sections were cut through central nucleus of the amygdala and basolateral amygdala regions by using a cryostat (Microm, Germany) and stained with thionin for

light microscopic examination. The position of the cannulas was examined on the basis of the stereotaxic atlas of Paxinos and Watson (1982). Only proper placements were included into the study.

2.5. Drugs

The following drugs were used: Ketamine HCl (Ketalar, Parke-Davis, gift from Eczacıbaşı, Turkey), chlorpromazine HCl (Largactil, gift from Eczacıbaşı, Turkey), atropine sulfate (Sigma, St. Louis, MO, USA), pirenzepine dihydrochloride (Sigma, St.Louis, MO), methoctramine (gift from Dr. C. Melchiorre, Italy), heparin sodium (Liquemine, gift from Roche, Turkey). All drugs were dissolved and diluted in saline.

2.6. Data analysis

The results were expressed as mean ± S.E.M. of 4–8 rats in each group. Mean arterial pressure was calculated as '1/3 pulse pressure + diastolic blood pressure'. The ID_{50} value of pirenzepine was calculated by probit analysis and the confidence limits were determined according to Litchfield and Wilcoxon (1949). Data were statistically evaluated using one-way analysis of variance (ANOVA) and Fisher's post-hoc test. Two-way ANOVA was used to compare the time–response curves obtained with different drugs and doses. The level of statistical significance was considered to be $P < 0.05$.

3. Results

The mean resting mean arterial pressure and heart rate in all animals ($n = 41$) were 106.0 ± 1.5 mmHg and 356.6 ± 8.7 beats/min. There were no significant differences between the pre-drug resting mean arterial pressure and heart rate for all groups. Saline, pirenzepine and methoctramine injected into the amygdala did not affect baseline mean arterial pressure and heart rate values significantly.

Carbachol (100 ng) administered into the lateral cerebral ventricle in animals pre-injected with saline into the central nucleus of the amygdala increased the mean arterial pressure and decreased the heart rate significantly ($P < 0.001$; Fig. 2). These cardiovascular responses appeared in 1 min, reached their maximum in 5 min and returned to the basal values within 15–20 min. The maximum change in mean arterial pressure was 31.8 ± 4.5 mmHg and in heart rate was 80.0 ± 12.2 beats/min. The animals exhibited behavioral changes such as restlessness and exploration within the first 3–5 s of carbachol injection.

Pirenzepine (10–75 nmol) injected into the central nucleus of amygdala inhibited carbachol-induced pressor responses dose-dependently ($P < 0.001$; Fig. 2). The ID_{50} value of pirenzepine that lead a 50% suppression in the peak pressor response to carbachol was calculated as 43.3 nmol (confidence interval: 16.8–111.8). The bradycardic

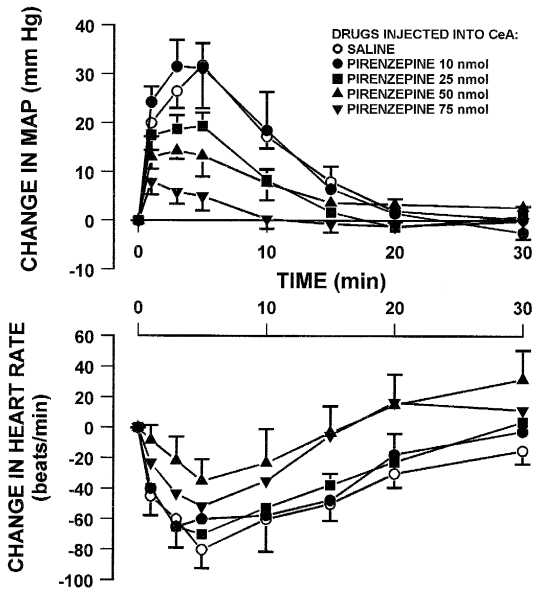

Fig. 2. The effect of i.c.v. injection of carbachol (100 ng) on mean arterial pressure (mmHg) and heart rate in conscious, unrestraint Sprague–Dawley rats pretreated with saline or pirenzepine (10, 25, 50 and 75 nmol) injected into the central nucleus of the amygdala (CeA). Each point represents the mean ± S.E.M. of 4–6 individual experiments.

responses to carbachol were also inhibited by pirenzepine, but no dose-dependency was observed, and 50 and 75 nmol doses suppressed carbachol-induced bradycardia to a similar extent ($P < 0.005$; Fig. 2). The injection of pirenzepine into the basolateral amygdala at a dose that inhibited carbachol-induced mean arterial pressure and heart

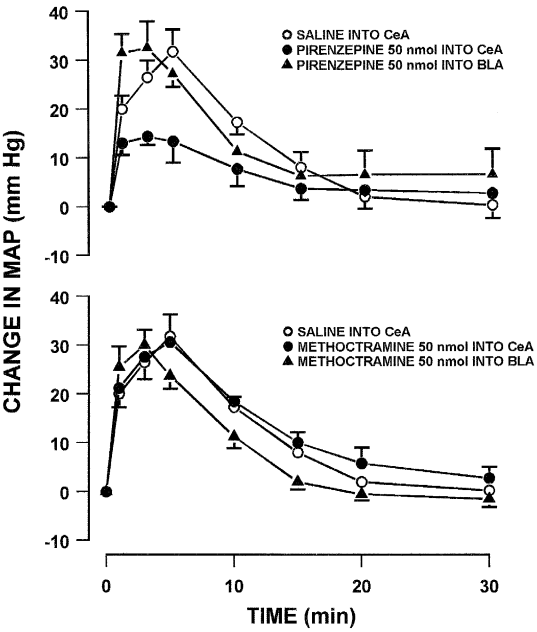

Fig. 3. The effect of i.c.v. injection of carbachol (100 ng) on mean arterial pressure (MAP, mmHg) in conscious, unrestraint Sprague–Dawley rats pretreated with saline injected into the central nucleus of the amygdala (CeA), pirenzepine (50 nmol) or methoctramine (50 nmol) injected into CeA or basolateral amygdala (BLA). Each point represents the mean ± S.E.M. of 4–6 individual experiments.

rate changes when injected into the central nucleus of amygdala failed to exert any inhibition (Fig. 3). Methoctramine at a dose of 50 nmol injected into both the central nucleus of the amygdala and the basolateral amygdala did not cause any significant alteration in the responses (Fig. 3).

4. Discussion

The present data demonstrate that injection of carbachol into the lateral cerebral ventricles in conscious, unrestraint Sprague–Dawley rats evokes a pressor response associated with a decrease in heart rate. This i.c.v. carbachol-induced pressor response was found to be attenuated by the selective muscarinic M_1 receptor antagonist, pirenzepine (Hammer et al., 1980) when injected into the central nucleus of the amygdala, in a dose-dependent manner. However, the selective muscarinic M_2 receptor antagonist, methoctramine (Giraldo et al., 1988), could not inhibit the pressor response at a dose of 50 nmol, although the inhibitory effect of the same dose of pirenzepine was significant. As pirenzepine is approximately 10 times more potent at muscarinic M_1 and 20 times less potent at muscarinic M_2 receptors than methoctramine and both compounds are equipotent at M_3 and M_4 receptors (Dörje et al., 1991; Doods et al., 1993), the results of the present study suggest that muscarinic M_1 receptors mediated the above described carbachol-induced pressor response. This effect seems to be specific to the central nucleus of the amygdala since both pirenzepine (50 nmol) and methoctramine (50 nmol) were ineffective when injected into the basolateral amygdala.

The amygdala has been implicated in the control of several autonomic functions such as respiration and cardiovascular functions, including the regulation of blood pressure (Dampney, 1994; Davis et al., 1994). For instance, ablation of the amygdala is reported to delay the development of hypertension and to attenuate the exaggerated pressor responses to noise stress in spontaneously hypertensive rats (Galeno et al., 1982, 1984). Further, ablation of the central nucleus of the amygdala significantly attenuates i.c.v. carbachol-induced pressor responses in both anesthetized and conscious rats (Özkutlu et al., 1995). In our previous experiments, we also found that the pressor responses to electrical and carbachol-induced stimulation of the central nucleus of the amygdala were inhibited by the muscarinic receptor antagonists, atropine, pirenzepine and AF-DX 116 (11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one). Pirenzepine has been found to be more potent than AF-DX 116 and is equipotent to atropine in this respect (Aslan et al., 1995, 1997). These combined observations suggest that, at the level of the amygdaloid complex, cholinergic pathways acting via M_1 muscarinic receptors may play an important role in the regulation of blood pressure and in stress-induced hypertension.

The central cholinergic regulation of heart rate seems to be independent of that of blood pressure. For example, the heart rate changes produced by carbachol injection into the central nucleus of the amygdala are reported to be variable (Ohta et al., 1991). Furthermore, the electrolytic ablation of this nucleus does not alter i.c.v. carbachol-evoked bradycardia while the pressor responses are significantly inhibited by this intervention (Özkutlu et al., 1995). However, although this effect was not dose-dependent, 50 and 75 nmol doses of pirenzepine injected into the central nucleus of the amygdala suppressed the bradycardic effect of i.c.v. carbachol. Therefore, it may be concluded that M_1 receptors are at least partially involved in carbachol-induced changes in heart rate. The lack of an inhibitory effect of methoctramine supports this suggestion.

In conclusion, the present data support the view that M_1 muscarinic receptors seem to be the major subtype involved in the cardiovascular regulation (Barnes and Roberts, 1991; Polidori et al., 1991; Scheucher et al., 1991) mediated by central cholinergic pathways, and that the muscarinic M_1 receptors in the central nucleus of the amygdala are particularly important for this integration.

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