Clinical and Experimental Pharmacology and Physiology (1989) **16**, 19–24

CARDIOVASCULAR EFFECTS OF CENTRAL ADMINISTRATION OF β-ENDORPHIN IN RATS RECEIVING NEONATAL INJECTION OF MONOSODIUM GLUTAMATE

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(Received 31 May 1988; revision received 15 August 1988)

SUMMARY

1. The effects of intracerebroventricular (i.c.v.) and intracisternal (i.c.) injection of β-endorphin on arterial blood pressure (BP) in rats that received five intraperitoneal injections of monosodium glutamate (MSG) on alternate days in the first 10 days of life were studied.

2. β-endorphin administered into the lateral ventricles caused a prolonged elevation in BP, whereas i.c. injection of the peptide resulted in an even longer lasting reduction in BP. In the MSG-treated rat, the prolonged hypertensive effect of i.c.v. injection of β-endorphin was completely abolished, but the effect of i.c. injection of the peptide was the same as that in the control. Since MSG treatment destroyed selectively the structures around the third ventricle, it is suggested that these structures, including the arcuate nucleus, may be responsible for mediating the cardiovascular effects of β-endorphin.

3. The effects of central administration of β-endorphin were completely blocked by naloxone, which mainly antagonizes the actions of μ-receptor agonists and has no cardiovascular effects itself. The results suggest that μ-receptors may be involved in mediation of the effects of β-endorphin on the cardiovascular system and that β-endorphin in the brain may not exert a tonic influence on the cardiovascular functions.

Key words: blood pressure, β-endorphin, fourth ventricle, intracerebroventricular, intracisternal, monosodium glutamate, neonatal, rat, third ventricle.

INTRODUCTION

Administration of opioids into the brain results in alterations of cardiovascular functions (Feldberg & Wei 1978, 1979; Holaday 1982; Tse & Wong 1984), suggesting a possible involvement of opioid peptides in the central regulation of the cardiovascular system. The neuronal substrates involved have not been clearly revealed yet. In the chloralose-anaesthetized cat, intracerebroventricular (i.c.v.) injection of morphine causes tachycardia and a brief elevation in arterial blood pressure (BP) (Feldberg & Wei 1978). The effects were believed to result from an

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action on the wall of the third ventricle, as morphine produced the effects only when it was allowed to reach the third ventricle (Feldberg & Wei 1978). On the other hand, intracisternal (i.c.) injection of morphine to the chloralose-anaesthetized cat results in bradycardia and a reduction in blood pressure (Feldberg & Wei 1978). The neuronal substrates involved were believed to be located on the dorsal surface of the medulla since topical application of morphine to this area produced similar effects (Feldberg & Wei 1978).

Neonatal treatment of monosodium glutamate (MSG) destroys selectively the structures located in a paramedian plane and roof and floor of the third ventricle and some nuclei in the hypothalamus including the β-endorphin-containing arcuate nucleus (Olney 1969). It is therefore likely that the cardiovascular responses to β-endorphin administered to the third ventricle would be altered as the structures surrounding it are believed to be involved in mediation of opioid action. In this study, the effects of central administration of β-endorphin on BP in the pentobarbital-anaesthetized rat that had received injection of MSG neonatally were investigated. The aim was to obtain more information on the neuronal substrates involved in the mediation of β-endorphin-induced alterations of cardiovascular functions and possible involvement of this peptide in the central regulation of the cardiovascular system.

METHODS

The methods of Nemeroff *et al.* (1977) and Greeley *et al.* (1978) were adopted for administration of MSG. On alternate days in the first 10 days of life, female Sprague-Dawley neonatal rats were injected intraperitoneally with either five doses of monosodium-L-glutamate (Sigma) at a dose of 4 mg/g bodyweight dissolved in 0.9% NaCl solution or five doses of 3.4% NaCl solution as an isosmotic control. All animals were weaned at 21 days of age. At 8–10 weeks of age when bodyweight was in the range of 250–300 g, the rats were anaesthetized with pentobarbital sodium at a dose of 60 mg/kg intraperitoneally. The animal was then tracheotomized and the intubated cannula connected to an artificial respirator. Respiratory rate was synchronized with that of the rat (60–80 per min) with a tidal volume of 1.6–2 ml. The right femoral artery was cannulated for the measurement of BP by a Statham pressure transducer connected to a chart recorder (Gould).

The rat was mounted on a Kopf stereotaxic instrument. To access the lateral ventricle, the dorsal surface of the skull at the frontal and parietal regions were exposed by splitting the scalp longitudinally. A trephine hole was made with a hand drill at the bregma and a small stainless steel implant was fixed into the lateral ventricle. The co-ordinates for the site of the lateral ventricle were: AT 0.5 mm, ML 1.0 mm and DV 7.5–8.0 mm below the surface of the skull according to Skinner (1971). In another group of rats, the fourth ventricle was accessed by an i.c. puncture with a small stainless steel needle which was fixed by a stereotaxic instrument.

The rat was allowed to stabilize for 20–25 min. Then, β-endorphin (Peninsula Lab.) at doses of 3 and 9 nmol/kg in 10 μl or saline alone as control was injected into the lateral or fourth ventricles of the rat which had received either 10 μl of saline solution or naloxone (DuPont) at a dose of 375 nmol/kg via the same route 5 min previously. Naloxone at this dose has been shown previously to cause no significant alterations in BP (Lee 1988). BP was continuously monitored throughout the experiment for 80 min.

The injection sites were marked after every experiment by the injection of 0.1 ml thionin blue (0.1%) *in situ*. The hypothalamus was dissected according to the method described by Glowinski and Iversen (1966). The brain was then removed. Final histological verification of the injection sites and identification of hypothalamic lesions were made on frozen section (60 μm, transverse)

stained with haematoxylin and eosin for the former and neutral red for the latter, respectively. Data from animals with wrong injection sites were discarded.

Analysis of variance (split plot design) was used to compare the difference in time courses of changes in BP between different groups.

RESULTS

In accordance with the observations in the previous studies (Olney *et al.* 1969; Nemeroff *et al.* 1977), the MSG-treated rats exhibited all the abnormalities characteristic of MSG syndrome, including ($n = 49$) stunted growth (100%), obesity (94%), tail automutilation (27%) and optic tract degeneration (100%). The density of neurons in the structures surrounding the third ventricle, especially in the arcuate nucleus, was greatly reduced (Fig. 1), indicating that they had, at least partly, been destroyed.

The effects of β-endorphin on BP in MSG-treated rats are shown in Figs 2 and 3. In the control rat, i.c.v. injection of β-endorphin at the dose 9 μmol/kg caused a significant increase in BP, which reached a plateau at 40 min after injection and lasted for well over 80 min, whereas i.c. injection of the same dose of the peptide resulted in an even longer lasting and significant reduction in BP. In the MSG-treated rat, both i.c.v. and i.c. injection of β-endorphin at the dose of 9 μmol/kg led to a significant reduction in BP, the magnitude and duration of which were similar to that of the control group receiving i.c. injections of the same peptide. The effects of β-endorphin were completely blocked by pretreatment of naloxone in all cases.

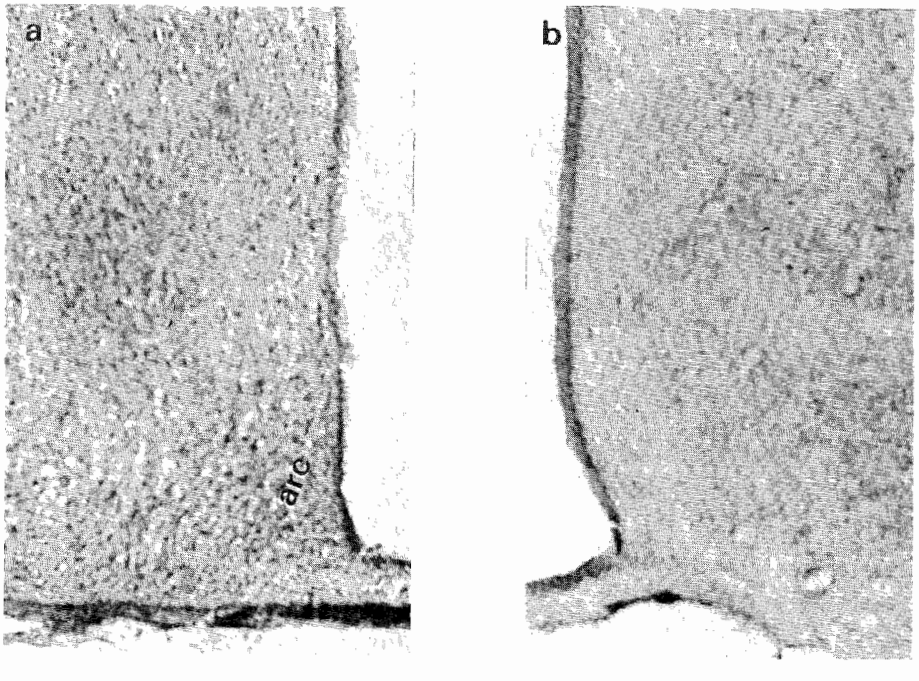

Fig. 1. Hypothalamus of an adult rat that received neonatal treatment of MSG (×196). (a) Control rat. (b) MSG-treated rat. Note the reduced neuronal density especially in the arcuate nucleus (arc).

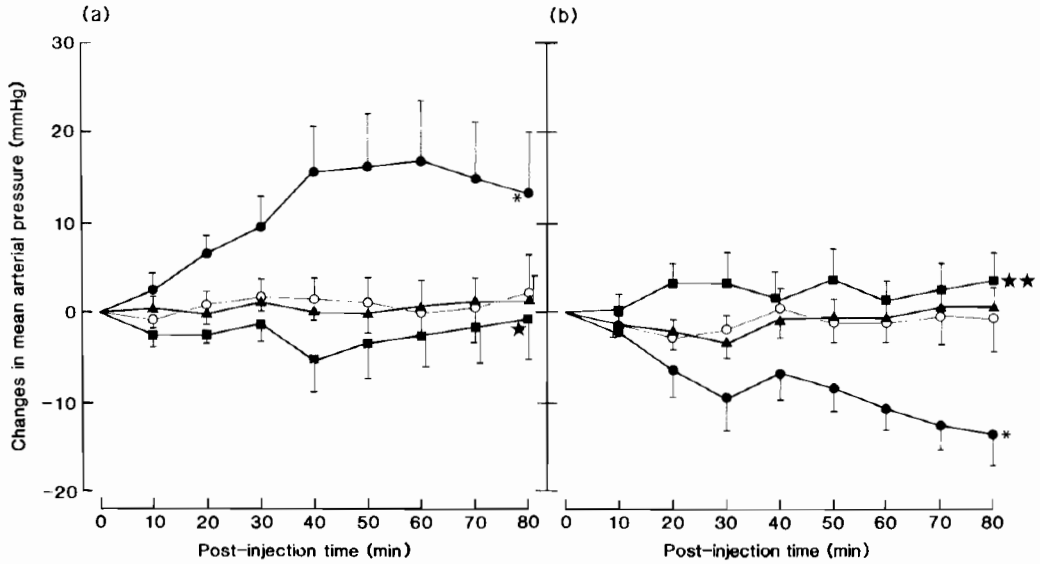

Fig. 2. Effects of i.c.v. injection of β-endorphin on BP in the MSG-treated rats. (a) Control rat group, (b) MSG-treated group, $n = 8$ in all groups. (▲) Saline; (○) β-endorphin 3 nmol/kg; (●) β-endorphin 9 nmol/kg; (■) β-endorphin 9 nmol/kg with pretreatment of naloxone 375 μmol/kg. *,** signify statistical difference to the corresponding saline-injected control groups by analysis of variance (split plot design) to the levels $P<0.05$ and $P<0.01$, respectively. ★,★★ signify statistical difference to the corresponding groups injected with 9 nmol/kg of β-endorphin by analysis of variance (split plot design) to the levels $P<0.05$ and $P<0.01$, respectively.

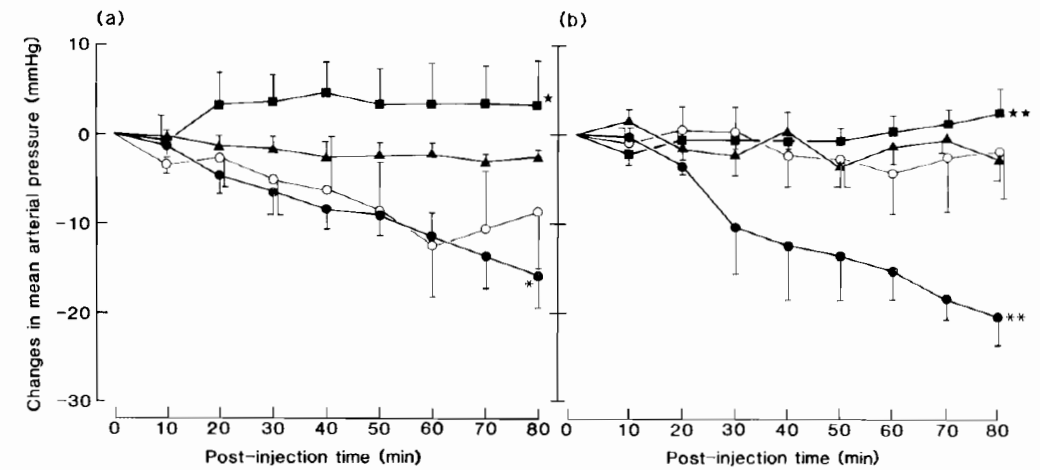

Fig. 3. Effects of i.c. injection of β-endorphin on BP in the MSG-treated rat. For description, see Fig. 2.

DISCUSSION

The results of the present study, that i.c.v. injection of β-endorphin in the pentobarbital-anaesthetized rat caused a prolonged increase in BP (Fig. 2), are in agreement with the previous finding that injection of morphiceptin (Holaday 1982) or β-endorphin (Tse & Wong 1984)

directly into the third ventricle produced a long-lasting elevation in BP in the pentobarbital-anaesthetized rat. The findings are, however, at variance with the results in the chloralose-anaesthetized cat, in which i.c.v. injection of both morphine (Feldberg & Wei 1978) and β-endorphin (Feldberg & Wei 1979) increased the heart rate (HR), and the tachycardiac effect of morphine (Feldberg & Wei 1978), but not β-endorphin, was accompanied by a brief elevation of BP (Feldberg & Wei 1979). An i.c. injection of β-endorphin in these rats also resulted in a long-lasting fall in BP (Fig. 3). The result is in keeping with the finding that in the chloralose-anaesthetized cat both morphine (Feldberg & Wei 1978) and β-endorphin (Feldberg & Wei 1979) reduced BP when administered via the same route. However, i.c. injection of morphine in the pentobarbital-anaesthetized rat did not always lead to a reduction in BP (Holaday 1982). The discrepancies may be related to the anaesthetics, ligands and animals used in different studies.

Neonatal treatment with MSG abolished completely the hypertensive effect of i.c.v. injection of β-endorphin; instead, the response was a prolonged reduction in BP, a characteristic response to i.c. injection of the peptide (Figs 2, 3). Since neonatal treatment of MSG destroys the structures on the wall of the third ventricle (Olney 1969), the result may be taken as a suggestion that these structures may mediate the effects of β-endorphin on BP. This is in agreement with the result of Feldberg and Wei (1978) that morphine produced its cardiovascular effects — an increase in HR and a brief elevation in BP — only when it was administered to the third ventricle, but not to the lateral or fourth ventricle, indicating that it is the structures associated with the third ventricle that mediate the effects of the opioid. Together with the finding that in the anteroventral third ventricle region of the brain are vasoconstrictor and vasodilator sites (Mangiapane & Brody 1987), the results of the present study and that of Feldberg and Wei indicate that the wall of the third ventricle may contain neuronal substrate(s) mediating the action of humoral substances. Special mention is made of the arcuate nucleus, which was completely destroyed by the MSG-treatment, as it has been shown to possess pre-opiomelanocortin (POMC)-producing neurons with POMC-containing fibres in many parts of the brain, including the periaqueductal grey (see Akil *et al.* 1984; Khachaturian *et al.* 1985), a structure shown to be involved in the regulation of cardiovascular functions (Hilton & Redfern 1986). Further studies are, however, needed to determine the specific structure(s) involved in the mediation of the actions of these opioids and other humoral substances.

In agreement with our previous findings (Ise & Wong 1984) that naloxone, which itself does not cause any significant change in BP, blocked completely the effects of β-endorphin injected centrally both in control and MSG-treated rats. The results suggest that the endorphinergic system in the brain, if there is one, does not exert a tonic influence on the cardiovascular functions. The complete blockade of the effects of β-endorphin by naloxone, a predominant μ-receptor antagonist, also suggest that μ-receptors are probably involved in mediating the effects of β-endorphin on the cardiovascular system. Verification of these suggestions needs further study.

ACKNOWLEDGMENTS

We thank Dr P. P. H. Chow, Dr W. S. O, Mr C. P. Mok and Mrs Cindy Li for advice on histological examination and on photographing, technical assistance and typing the manuscript, respectively. The study was supported by Hong Kong University Research Grants. Naloxone was kindly supplied by DuPont Pharmaceutical Co.

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