Br. J. Pharmac. (1981), **74**, 105–109

POTENTIATION BY NALOXONE OF PRESSOR REFLEXES

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1 The effect of intravenous naloxone, an opiate antagonist, was studied on the pressor responses elicited by stimulation of afferent nerves (vagus and laryngeal superior nerves) in anaesthetized dogs.

2 Although naloxone (0.1 mg/kg i.v.) alone failed to modify basic blood pressure, the pressor responses induced by stimulation of either the vagus or laryngeal nerve were potentiated by naloxone.

3 Morphine (0.2 mg/kg i.v.) suppressed these two cardiovascular responses. These depressor effects of morphine were reversed by subsequent injection of naloxone (0.1 mg/kg i.v.).

4 The results suggest the involvement of endogenous opiate peptides in pressor reflexes elicited by stimulation of the afferent nerves.

Introduction

Brain neurotransmitters and especially monoamines have been implicated in the mechanism of experimental arterial hypertension (Chalmers, 1975; Haeusler, 1976). More recently, it was suggested that morphinomimetic peptides could play an important inhibitory role in central control of blood pressure (Laubie, Schmitt, Vincent & Remond, 1977; Feldberg & Wei, 1978; Freye & Arndt, 1978; Bolme, Füxe, Agnati, Bradley & Smythies, 1978; Arndt & Freye 1979b; Bellet, Elghozi, Meyer, Pernollet & Schmitt, 1980) or sympathetic tone (Farsang & Kunos, 1979). However, it is generally accepted that the opiate antagonist naloxone (Sawynok, Pinsky & Labella, 1979) alone does not affect blood pressure or heart rate (Farsang & Junos, 1979), although cardiovascular effects of nalone have been described in intact animals, especially at very high doses: Feria, Armijo, Mediavilla & Florez (1975) found that naloxone (at doses up to 3 mg/kg i.v.) induced a dose-dependent hypotensive response in rats anaesthetized with thiopentone plus urethane, whereas Feria, Boada & Alvarez (1980) have shown that naloxone (5 mg/kg i.v.) potentiated the pressor response elicited by noradrenaline injections. In the present study the effects of naloxone on pressor responses to afferent nervous stimulation (Korner, 1971; Paintal, 1973; Sato & Schmidt, 1973) were studied in anaesthetized dogs. In previous studies, it was shown that these pressor reflexes were reduced by (±)-propranolol, but not by (+)-propranolol or clonidine (Montastruc, Montastruc & Moatti, 1978a; Montastruc, Montastruc, Moatti & Mauco, 1978b).

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Methods

Animals

Eleven dogs of either sex (mean weight 17.6 kg) were used. Before the experiments they were kept in the animal room maintained at a temperature of 19 ± 1°C and were fed a dry commercial food (U.A.R. Villemoison sur Orge, France) once daily at 09 h 00 min. Drinking water was always available. All experiments were done between 08 h 30 min and 12 h 00 min.

Pressor responses to afferent nervous stimulation

The dogs were anaesthetized with urethane (1 g/kg i.v.). The trachea was intubated, the dogs were curarized (gallamine, 2 mg/kg i.v.) and artificially respired with an Ideal Palmer Pump. As in previous experiments (Laporte & Montastruc, 1957; Baïsset, Laporte & Montastruc 1959; Baïsset & Montastruc, 1964) urethane was chosen because it increased vascular reactions (especially pressor responses to nervous stimulation), and gallamine prevented the variations of the carotid arterial pressure induced by respiratory irregularities without inducing ganglioplegy (Laporte & Montastruc, 1957). However, gallamine does have some vagolytic properties, in keeping with which a small tachycardia

0007-1188/81/09 0105-05 $01.00

without modification of the carotid arterial pressure was noted in some experiments. Adequate anaesthesia was maintained by injection of 0.10 g of urethane every half hour.

A previously described technique (Laporte & Montastruc, 1957; Baïsset *et al.*, 1959; Baïsset & Montastruc, 1964) was used for the stimulation of the sensory fibres of the vagus nerve (in the neck, caudal to the nodose ganglion) and the superior laryngeal nerve. In brief, after dissecting, the nerves were sectioned and the central end immersed in a pool of paraffin oil (38°C). The central end of the nerve was stimulated via bipolar stainless steel electrodes. The stimuli consisted of 15 s trains of rectangular pulses (40 V), supramaximal intensity, duration 0.1 ms, frequency 5 Hz for the vagus and 10 Hz for the superior laryngeal nerve. These experimental conditions permit stimulation of nociceptive fibres (Aδ and C) as previously described in cats by Laporte & Montastruc (1957). These authors have shown that Aα fibres have only depressor action. Aδ fibres have pressor or depressor properties according to the stimulation frequency, whereas stimulation of C fibres induced only pressor effects. The superior laryngeal nerve contains both motor and sensory fibres. However, the sensory fibres in the vagus and the superior lanryngeal nerve also innervate a variety of visceral receptors: in addition to nociceptive fibres, aortic baroreceptors and chemoreceptors were stimulated (Korner, 1971; Paintal 1973; Sato & Schmidt, 1973).

In a first series of experiments (experiment I), afferent stimulation was performed before (basic conditions) and 15 and 60 min after administration of naloxone. In another series of experiments (experiment II), the effects of morphine on pressor response to nervous stimulation were studied. Afferent stimulation was performed before (basic conditions) and 15 min after morphine.

Measurement of blood pressure

Mean blood pressure was recorded in the left femoral artery of the dog by means of a Statham P 23 Db pressure transducer on a channel of a Philips recorder. The femoral artery was cannulated according to the method of Sedlinger (1953). The electrocardiograph and heart rate were also recorded by means of a tachocardiometer (Philips).

Drugs

Naloxone hydrochloride (Endo Laboratories) was dissolved in 0.9% w/v sodium chloride solution (saline). After checking the induced pressor response (experiment I), naloxone was injected and nervous stimulation performed 15 and 60 min later. In experiment II, morphine hydrochloride (commercial ampoules; obtained from Meram Laboratories; Melun, France) was also injected intravenously (0.2 mg/kg i.v.) after nerve stimulation. In the two series of experiments, naloxone was used at a dose 0.1 mg/kg (single injection) chosen in accordance with the results of Laubie *et al.* (1977) and Laubie, Schmitt & Vincent (1979).

Statistical evaluations

Statistical analysis was performed using Student's t test for paired comparisons. When the P value was greater than 0.05, the difference was not considered to be significant. The mean values of blood pressure are reported with standard error of the mean ($\pm$ s.e.mean).

Results

Experiment I: Effect of naloxone on pressor responses to nervous stimulation (Figure 1)

The basic (before nerve stimulation) blood pressures were 214 ± 21 mmHg (systolic) and 118 ± 17 mmHg (diastolic). Under these conditions afferent stimulation of the central end of each nerve induced a significant increase in both systolic and diastolic blood pressure values: for example, the increase in systolic blood pressure was $+61 \pm 11$ ($n = 6$) and 55 ± 15 ($n = 6$) mmHg for the vagus and superior laryngeal nerves respectively.

Subsequent injection of naloxone (0.1 mg/kg i.v.) did not change the values of the basal blood pressure. However, 15 min later, the pressor nervous response was significantly ($P < 0.05$) greater than before naloxone; for example, the increase in the systolic values was $+87 \pm 15$ ($n = 6$) for the vagus and $+82 \pm 13$ ($n = 6$) for the superior laryngeal nerve. One hour after the injection of naloxone, the pressor responses elicited by stimulation of the two nerves were identical to those observed under control (pre-naloxone) conditions, probably because of the short half life of naloxone.

Experiment II: Effect of morphine on pressor responses to nerve stimulation (Figure 2)

In this series of experiments basic values (before nerve stimulation) of blood pressures were 210 ± 18 mmHg (systolic) and 120 ± 15 mmHg (diastolic). Afferent stimulation of the central end of the nerves significantly ($P < 0.05$) increased systolic blood pressure: $+58 \pm 12$ mmHg for the vagus ($n = 5$) and $+53 \pm 14$ mmHg for the superior laryngeal nerve ($n = 5$) (systolic values).

Morphine, at the dose used (0.2 mg/kg i.v.), failed

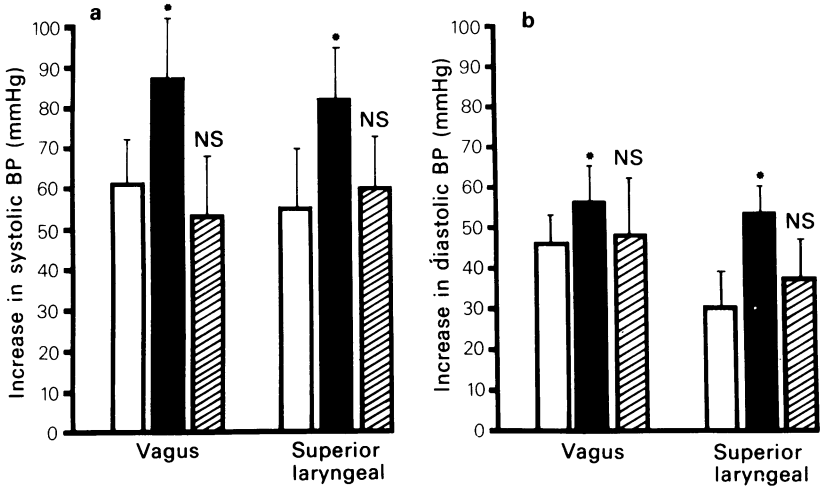

Figure 1 Effect of naloxone (0.1 mg/kg i.v.) on the pressor responses elicited by afferent nervous stimulation in the anaesthetized dog. The increase in systolic (a) and diastolic (b) blood pressure (mmHg) induced by afferent nervous stimulation under three experimental conditions is shown: open columns: before naloxone; solid columns: 15 min after naloxone; hatched columns: 60 min after naloxone. Statistical evaluations were made for each nerve using Student's t test for paired comparisons. $^*P < 0.05$; NS = not significant. Mean values are shown; vertical lines indicate s.e.mean.

to modify the basal blood pressure. However, the pressor reflexes were significantly ($P < 0.05$) decreased after morphine: $+10 \pm 3$ mmHg for the vagus ($n = 5$) and $+2 \pm 1$ mmHg for the superior laryngeal nerve ($n = 5$). Subsequent injection of naloxone (0.1 mg/kg i.v.) re-established the two pressor reflexes.

Discussion

Afferent stimulation of vagus and superior laryngeal nerves induced pressor responses by stimulation of nociceptive and visceral fibres (Laporte & Montastruc, 1957; Baïsset *et al.*, 1959; Baïsset & Montrastruc, 1964). These cardiovascular reflexes are related to an increase in sympathetic tone (for references see introduction, and Montastruc *et al.*, 1978b). Our results show that naloxone increased and morphine decreased the magnitude of these nervous cardiovascular reflexes. In contrast, naloxone failed to modify the pressor response elicited by sino-aortic denervation in dogs (Montastruc & Montastruc, 1981).

Naloxone is a potent antagonist of morphine and of opioid peptides without exhibiting other pharmacological properties, at least at the doses used in the present study (Laubie *et al.*, 1977; 1979; Sawynok *et al.*, 1979). Since this drug can be used to provide indirect evidence for the release of opiate peptides (Akil, Madden, Patrick & Barchas, 1976; Dashwood & Feldberg, 1980), it is tempting to speculate that afferent nerve stimulation induces release of some endogenous opiate peptides. This involvement of morphinomimetic endogenous agents was confirmed by the fact that naloxone reversed the morphine-induced depressor effect on afferent nerve stimulation (vagus and laryngeal superior nerve).

Our results are in agreement with the recent findings of Dashwood & Feldberg (1980) in the cat who found that injections of naloxone produced a pressor response after at least two of the following surgical procedures: tying sinus nerves, removing stellate ganglia, cutting vagi, or evisceration.

Naloxone was shown to antagonize the hypotensive effect of endotoxin (Holaday & Faden, 1978; Janssen & Lutherer, 1980), inhalational anaesthetics (Arndt & Freye, 1979a, b) clonidine (Farsang & Kunos, 1979; Farsang, Ramirez-Gonzalez, Mucci & Kunos, 1980), α-methyldopa (Farsang *et al.*, 1980), spinal shock in animals (Holaday & Faden, 1980) and haemorrhagic shock in anephric cats (Feuerstein, Alicam & Bergman, 1980). Similar results were obtained in irreversible shock in man (Tiengo, 1980). According to these papers, it would be possible to interpret our results as being a consequence of the reversal of anaesthesia by naloxone. We disagree with such an explanation since we failed to observe any symptoms of inadequate anaesthesia after injection of naloxone and others have failed to obtain reversal of anaesthesia with naloxone, as discussed by Pokorski, Grieb & Wideman (1981). These results and the present paper suggest the involvement of an endogenous opiate component in the nervous pathways controlling the sympathetic tone.

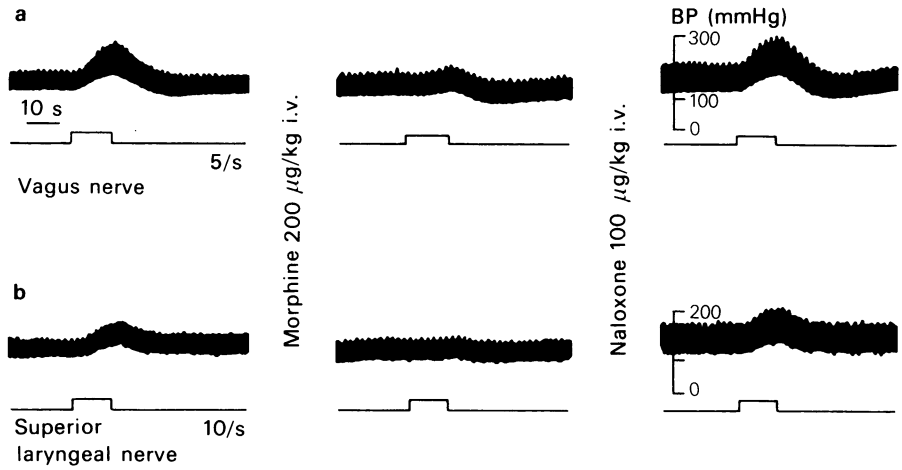

Figure 2 Effect of morphine (0.2 mg/kg i.v.) and naloxone (0.1 mg/kg i.v.) on pressor responses to stimulation of vagus (a) and superior laryngeal (b) nerves in the anaesthetized dog. The depressor effects of morphine were reversed by naloxone.

In conclusion, these experiments suggest the involvement of endogenous opioid substances in the pressor responses elicited by afferent stimulation of vagus or superior laryngeal nerves. Previous findings from our laboratory have shown the involvement of central adrenergic mechanisms in these reflexes since centrally injected (±)-propranolol and β-blocking agents were able to suppress these pressor responses (Montastruc *et al.*, 1978b). This paper and the present experiments could suggest an inhibitory role of endogenous opiates on the catecholamines released by electrical stimulation of nerves. Naloxone might suppress this inhibitory control, thus permitting an increase in the release of the adrenergic transmitter. This conclusion agrees with the well known opiate presynaptic inhibitory control of the release of noradrenaline (Langer, 1977).

The authors wish to thank Professor Yves Laporte (Laboratoire de Neurophysiologie, Collège de France, Paris, France) for help and encouragement during the preparation of the manuscript. They gratefully acknowledge the excellent technical assistance of Mrs Gayraud and Mr Bernou. The figures were prepared by Mrs Corbières and Mr Raoul. Naloxone was a generous gift from Dr L.J. Steinke (Endo Laboratories, New York). Correspondence to P.M. please.

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(Received February 24, 1981.
Revised May 22, 1981.)