Does $\beta$-endorphin contribute to the central antihypertensive action of $\alpha$-methyldopa in rats?

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Summary
1. Opioid peptide involvement in the fall in blood pressure resulting from central $\alpha$-receptor stimulation has been investigated.
2. In conscious renal hypertensive rats (two-kidney, one clip Goldblatt model), $\alpha$-methyldopa (100 mg/kg intraperitoneally) produced a long-lasting fall in blood pressure which was partially attenuated by pre-treatment with naltrexone (5 mg/kg subcutaneously).
3. Unilateral injection of $\alpha$-methyl-noradrenaline (50 nmol) into the nucleus tractus solitarii of normotensive, urethane-anaesthetized rats induced a fall in blood pressure and heart rate. This fall was prevented by pretreatment with naloxone, either 1 mg/kg subcutaneously or 1 and 10 ng administered directly into the nucleus.
4. Pretreatment with antiserum to $\beta$-endorphin (1:50 dilution), applied locally, also blocked the depressor response induced by the catecholamine.
5. These results suggest that the fall in blood pressure observed after $\alpha$-methyldopa and its active metabolite $\alpha$-methylnoradrenaline involves a $\beta$-endorphin-like peptide; a possible site of action is the nucleus tractus solitarii.

Key words: $\beta$-endorphin, $\alpha$-methyldopa, $\alpha$-methylnoradrenaline, opiates.

Abbreviation: NTS, nucleus tractus solitarii.

Introduction
$\beta$-Endorphin applied locally to the nucleus tractus solitarii (NTS) of the brain stem results in a decrease in blood pressure and heart rate [1]. The NTS is also a site of action of catecholamines and the centrally acting hypotensive agents clonidine and $\alpha$-methyldopa [2-4]. In conscious, spontaneously hypertensive rats (SHR), the decrease in blood pressure and heart rate produced by intravenous clonidine and $\alpha$-methyldopa is reversed by naloxone [5], indicating that central $\alpha$-receptor stimulation involves the activation of opiate receptors. Since naloxone and clonidine appear not to interact with the same receptor site, it was suggested that clonidine and $\alpha$-methyldopa induce the release of an endogenous opiate, which is involved in the central control of sympathetic tone [6]. More recently it has been reported that clonidine and $\alpha$-methylnoradrenaline, the latter an active metabolite of $\alpha$-methyldopa, induce the release of a peptide with $\beta$-endorphin-like immunoreactivity from brain-stem slices of SHR, but not from their normotensive, genetically matched, Wistar-Kyoto control rats [7]. This response is specific for the active L isomer of $\alpha$-methylnoradrenaline and is reduced by yohimbine. On the basis of these findings, it was speculated that the release of $\beta$-endorphin-like material in the brain stem contributes to the antihypertensive action of central $\alpha$-receptor stimulants in the SHR [7]. In this study a possible interaction between catecholamines and endorphins has been investigated in an experimental model of hypertension, and the NTS as a possible site of this effect has been examined.

Methods
Male Wistar rats (Cpb, TNO, Zeist, The Netherlands) were used. Renal hypertension was induced by the application of a solid silver clip (0.2 mm internal diameter) to the left renal artery of rats weighing 125–135 g (two kidney, one clip Goldblatt model) [8]. The control group was
subjected to the same operative procedure, but no clip was applied. The rats were used 3–4 weeks after operation, when under light ether anaesthesia a catheter was placed in a tail artery, tunneled under the skin and exteriorized at the scruff of the neck. The animals were allowed several hours to recover from the effects of the anaesthesia, before drug administration and blood pressure recordings commenced. Arterial pressure and heart rate were recorded from the tail artery catheter by means of a Statham P23-AC gauge transducer and displayed on a Grass polygraph.

For direct application of drugs into the NTS, rats weighing 200–250 g were anaesthetized with urethane (1.4 g/kg). The rat was placed in a stereotactic head holder and the head fixed to an angle of 45° downward. After exposure of the lower brain stem, unilateral micro-injections into the NTS were given through a glass cannula (outer diameter 60 μm), in a volume of 0.4 μl, which was delivered in 10 s. After experimentation the correct position of the injection sites was confirmed by histology. Blood pressure and heart rate were measured directly from a carotid artery catheter.

The following drugs were used: (−)α-methyl-dopa, (+)α-methylnoradrenaline, naloxone, antiserum to β-endorphin, and control rabbit serum. All agents were dissolved in, or diluted with, sodium chloride solution (154 mmol/l: saline).

Results

Effect of (−)α-methyldopa in renal hypertensive rats

The basal mean blood pressure of the conscious renal hypertensive rats ranged from 180 to 230 mmHg. α-Methyldopa (100 mg/kg intraperitoneally) induced a gradual decrease in blood pressure, which reached a maximum at about 2.5 h after administration, when the change was −60 mmHg. An initial increase in heart rate occurred, which persisted until about 60–90 min after administration [3].

The opiate antagonist naloxone (5 mg/kg subcutaneously) did not affect either the blood pressure or heart rate of the renal hypertensive rats. However, when it was administered 15 min before α-methyldopa, the decrease in blood pressure induced by the amine was both delayed and significantly (P < 0.05) attenuated from 2 h onwards (Fig. 1). The changes in heart rate induced by α-methyldopa were not affected by naloxone.
Opiates and the hypotensive effects of α-methyldopa

Bradycardia caused by α-methylnoradrenaline. However, a larger dose (10 ng) of the opiate receptor antagonist completely blocked both the fall in blood pressure and heart rate.

Microinjection of antiserum to β-endorphin (1:50 dilution) into the NTS, 10 min before α-methylnoradrenaline (50 nmol), blocked the fall in blood pressure, being about −24 mmHg after pretreatment with normal rabbit serum and −5 mmHg after β-endorphin antiserum. Heart rate decrease was reduced.

Discussion

In this study the possible involvement of an opioid peptide in the antihypertensive effect resulting from central α-receptor activation has been investigated. It has been reported that the antihypertensive action of clonidine and α-methyldopa in SHR is mediated by a β-endorphin-like peptide, which is released by these centrally acting α-agonists [6, 7]. Such an interaction also appears to be present in the renal hypertensive rat (two kidney, one clip Goldblatt model), since the subcutaneous administration of naltrexone partially antagonized the fall in blood pressure induced by α-methyldopa, injected intraperitoneally. This interaction, however, appears to be absent in the normotensive pentobarbitone-anaesthetized rat [9], although in that publication [9] the authors assumed that all opioid peptides produce a blood pressure increase.

Microinjection of α-methylnoradrenaline into the NTS produced a rapid and marked fall in blood pressure, which was accompanied by bradycardia. Both the depressor response and fall in heart rate were naloxone-reversible, the opiate antagonist being administered subcutaneously or directly into the NTS. These findings suggest that the activation of α-receptors in the NTS by α-methylnoradrenaline, to induce a fall in mean blood pressure, requires the stimulation of opiate receptors.

Antiserum to β-endorphin blocked the depressor response and reduced the bradycardia resulting from α-methylnoradrenaline administration. It is possible that the catecholamine is releasing a β-endorphin-like peptide to cause the fall in blood pressure and heart rate. However, it seems improbable that the opioid peptide involved is β-endorphin, since although β-endorphin produces a decrease in blood pressure and heart rate upon microinjection into the NTS, the time courses of these changes are much slower than those of α-methylnoradrenaline [1]. Perhaps a shorter fragment of β-LPH is involved, with which the antiserum may cross-react.

In conclusion, it appears that a β-endorphin-like peptide is involved in the hypotensive effect resulting from central α-receptor stimulation. The NTS is a possible site of this action.

References