



Farsang *et al.* (2) have shown the existence of an opioidergic component in the antihypertensive action of clonidine in spontaneously hypertensive models of rat; a similar mechanism is either absent or inactive in the normotensive Wistar Kyoto rats.

The objective of the present study was to investigate the role of opioidergic component in the antihypertensive action of clonidine in albino normotensive and DOCA-salt hypertensive models of rats.

## MATERIAL AND METHODS

Female albino rats weighing 200-300 g were used.

### *Preparation of hypertensive models (renal DOCA-salt hypertensive model) :*

Under ether anaesthesia the capsule of left kidney was removed carefully without disturbing the suprarenal gland and the kidney was exposed. The renal artery was ligated and the left kidney was cut off.

The incision was closed by Michael clip. To prevent infection, ampicillin was administered intramuscularly (50 mg/day for 3 days). The unilaterally nephrectomized rats were given deoxycorticosterone acetate (DOCA) subcutaneously 10 mg/week for 5 weeks and 1% sodium chloride solution for drinking purposes.

Renal DOCA-salt hypertensive and normotensive rats were anaesthetized with sodium pentobarbitone (40 mg/kg, ip). Tracheostomy was done. Jugular vein and the common carotid artery of left side were exposed. Polyethylene cannula was inserted into the jugular vein. Heparin (500 IU/kg) was administered intravenously. The common carotid artery was connected to Statham Pressure Transducer (model P 23 AA) recording on Sanborn Twin Viso Recorder (model 160-1300 B).

Agonists were injected through the jugular vein in a volume of 0.5 ml and flushed with 0.05 ml of normal saline. The antagonists were injected intraperitoneally. The agonists were administered 5 min after ip injection of naloxone or 30 min after ip injection of yohimbine. The results have been expressed as mean  $\pm$  S.E.M. The data were analysed by paired or unpaired student's 't' test for significance.

### **Drugs used**

Heparin (Gland Chemicals, Hyderabad), deoxycorticosterone acetate (Organon, Calcutta), ampicillin (Unique Pharmaceutical Lab., Bombay) clonidine (Sarabhai Chemicals, Baroda), morphine (Alembic Chemical Works, Baroda), yohimbine (Sigma, St. Louis) naloxone HCl (Endo Laboratories, New York) and Sodium pentobarbitone (Apetho Research Chemicals, Bombay).

## RESULTS

### Normotensive rats :

The mean arterial pressure of normotensive rats was  $135 \pm 5.2$  mmHg ( $n = 12$ ). At stable pressure intravenous administration of clonidine produced a biphasic effect, an initial transient pressor response which was not consistent but was occasionally seen with larger doses of clonidine. This was followed by a sustained depressor response which lasted for about 1 hr. The depressor response was dose-related. Clonidine administered at intervals of 1 hr in doses of 2.5, 5.0 and 10.0  $\mu\text{g}/\text{kg}$  produced a decrease in B.P.

Yohimbine (2 mg/kg, ip) or naloxone (2 mg/kg, ip) did not have any effect on the B.P.

Naloxone (2 mg/kg) did not modify the hypotensive effect of clonidine. However, yohimbine (2 mg/kg) significantly blocked the responses to clonidine (Fig. 1).

### NORMOTENSIVE RATS.

CLONIDINE  
 YOHIMBINE (2mg/Kg i.p.)+CLONIDINE  
 NALOXONE(2mg/Kg i.p.)+CLONIDINE

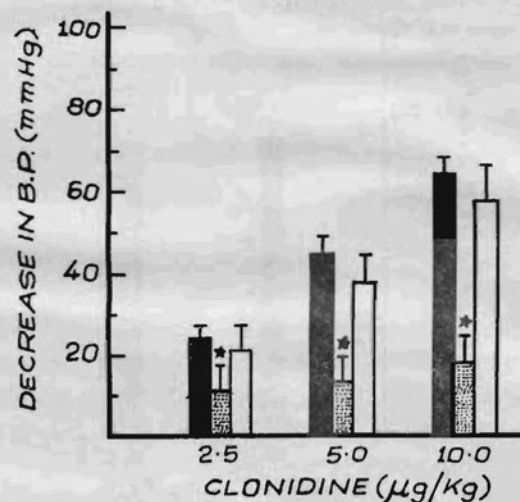


Fig. 1 : Effect of yohimbine and naloxone on clonidine-induced fall in blood pressure (B.P.) in anaesthetized normotensive rats. Black histograms depict responses to clonidine. Stippled and open histograms depict the hypotensive responses to clonidine in the presence of yohimbine and naloxone respectively. Vertical lines indicate S.E.M. ( $n=6$ ). Level of significance ( $P < 0.001$ ) is denoted by asterisk (\*).

### NORMOTENSIVE RATS

MORPHINE  
 NALOXONE (2mg/Kg i.p.)+MORPHINE  
 YOHIMBINE (1mg/Kg i.p.)+MORPHINE

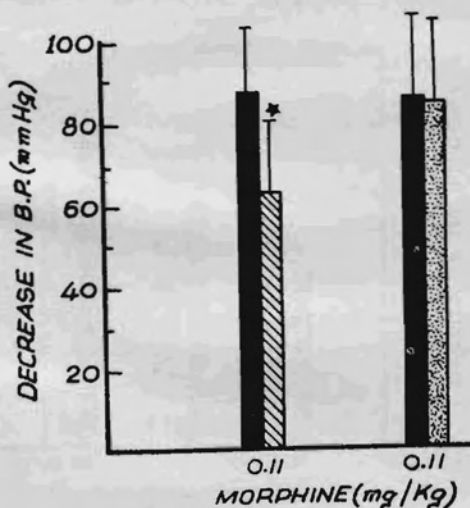


Fig. 2 : Effect of yohimbine and naloxone on morphine-induced fall in blood pressure (B.P.) in anaesthetized normotensive rats. Black histograms depict hypotensive responses to morphine. Hatched histograms depict hypotensive responses to morphine in the presence of naloxone while stippled histograms depict the hypotensive effect of morphine in the presence of yohimbine. Vertical lines indicate S.E.M. ( $n=6$ ). Level of significance ( $P < 0.05$ ) is denoted by asterisk (\*).

At stable pressure morphine (0.11 mg/kg) injected every 20 min produced a reproducible and significant decrease in B.P. Administered 5 min after naloxone (2 mg/kg), the hypotensive effect of morphine was significantly reduced. Yohimbine (1 mg/kg) did not modify the hypotensive effect of morphine (Fig. 2).

#### DOCA-salt hypertensive rats :

The mean arterial pressure of hypertensive rats was  $161.0 \pm 13.95$  mm Hg. At stable pressure, intravenous administration of clonidine produced a biphasic response, an initial pressor response which was not dose-dependent or consistent. This was followed by a sustained depressor response. The depressor response was dose-dependent and was significantly greater than that observed in normotensive rats.

Yohimbine (1 mg/kg) or naloxone (2 mg/kg) significantly reduced the hypotensive effects of 2.5, 5 and 10  $\mu$ g/kg clonidine (Fig. 3).

#### DOCA-SALT HYPERTENSIVE RATS

■ CLONIDINE  
 ▨ \*YOHIMBINE (1mg/Kg i.p.)+CLONIDINE  
 □ NALOXONE(2mg/Kg i.p.)+CLONIDINE

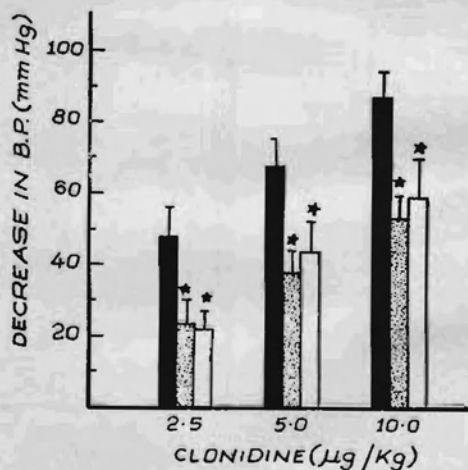


Fig. 3 : Effect of yohimbine and naloxone on clonidine-induced fall in blood pressure (B.P.) in anaesthetized renal DOCA-salt hypertensive rats. Black histograms depict responses to clonidine. Stippled and open histograms depict responses to clonidine in the presence of yohimbine and naloxone respectively. Vertical lines indicate S.E.M. ( $n=6$ ). Level of significance ( $P < 0.001$ ) is denoted by asterisk (\*).

#### DOCA-SALT HYPERTENSIVE RATS

■ MORPHINE  
 ▨ NALOXONE (2mg/Kg i.p.)+MORPHINE  
 ▩ \*YOHIMBINE (1mg/Kg i.p.)+MORPHINE

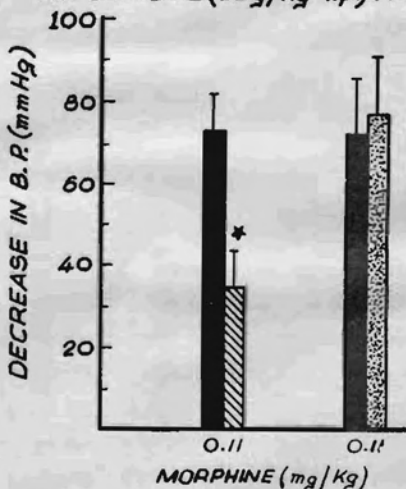


Fig. 4 : Effect of yohimbine and naloxone on the hypotensive effect of morphine in renal DOCA-salt hypertensive rats. Black histograms depict hypotensive responses to morphine. Hatched histogram depicts responses to morphine in the presence of naloxone and stippled histogram depicts responses to morphine in the presence of yohimbine. Vertical lines indicate S.E.M. ( $n=5$ ). The level of significance ( $P < 0.05$ ) is denoted by asterisk (\*).

Morphine (0.11 mg/kg, ip) reduced the B.P. which was significantly blocked by naloxone. Yohimbine did not modify the hypotensive effect of morphine (Fig. 4).

## DISCUSSION

In the present study clonidine produced a hypotensive effect in renal/DOCA-salt induced hypertensive rats. The effect of clonidine was blocked either by naloxone or yohimbine. Morphine also produced a hypotensive effect. This may partly be due to peripheral vasodilatation and partly due to activation of central opiate receptors (3,6). The effect of morphine was blocked by naloxone but not by yohimbine. Naloxone is a selective opioid-receptor antagonist, free of pharmacological actions of its own (also confirmed in the present study).

It is concluded that in the sequence of clonidine-induced hypotension activation of opiate-receptors occurs distal to the activation of alpha-adrenoceptors. It is likely that activation of opiate receptors through release of an endogenous opioid contributes to the centrally mediated hypotensive action of clonidine.

Naloxone did not modify the hypotensive effect of clonidine in the normotensive rats. This indicates that in the normotensive control rats, the opioidergic component postulated above, is either absent or inactive. Our results are in agreement with those of Farsang *et al.* (2) who have shown that in conscious and anaesthetized normotensive WKY rats, the reduction in B.P. and heart rate by clonidine were not influenced by naloxone, Rockhold and Caldwell (7) have shown that the destruction of nucleus tractus solitarius (NTS), abolishes the hypotensive and bradycardiac effects of clonidine in SHR but did not affect the hypotension and only partially reduced the bradycardia in normotensive WKY rats. Possibly a similar difference is present in animals used in the present study; this could suggest that the possible site of clonidine-induced opiate release is in a descending pathway originating in the NTS.

Physiological mechanisms controlling pain sensitivity and those involved in the regulation of B.P. are probably closely related as indicated by an opiate mediated decrease in pain sensitivity in various forms of hypertension (12). Thus the physiological control mechanisms involving endogenous opioids may be inactive under normal conditions but may become activated by the hypertensive process.

## ACKNOWLEDGEMENT

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